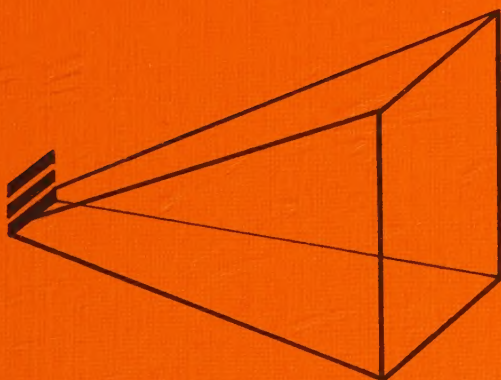


RAPID ROCK ANALYSIS AND MICROPROBE  
SCANNING OF DERMATOLOGICAL MATERIAL  
USING  
PROTON-INDUCED X-RAY AND  $\gamma$ -RAY EMISSION

Lars-Eric Carlsson





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av

Lars-Eric Carlsson

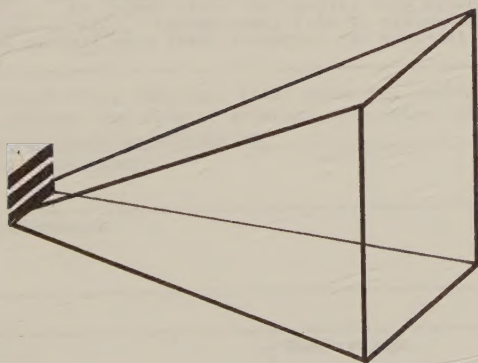
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| <p><b>Abstract</b> The proton-induced X-ray and <math>\gamma</math>-ray emission (PIXE-PIGE) technique has been applied to the elemental analysis of geological material. The elements with <math>Z=3,5,9</math> and <math>11</math> to <math>13</math> are detectable through <math>\gamma</math>-ray analysis and all heavier elements through X-ray analysis. Detection limits in the <math>2-100 \mu\text{g/g}</math> region were attained for many of the elements in a single 5 minute irradiation. /Nucl. Instr. Meth. <b>181</b> (1981) 531-537/</p> <p>The possibility of using the PIXE-PIGE technique for drill core analysis has been facilitated by designing a set-up for proton beam irradiation in air. Comparisons made with secondary-target mode X-ray fluorescence methods (XRF) showed similar detection limits for the two systems. The PIGE analysis, though, increases the elemental range compared with XRF and very advantageous behaviour of matrix corrections using the PIXE-PIGE method was illustrated. /Adv. X-ray Anal. vol.24 (1981) 313-321./</p> <p>By repeated analyses of international geological standards during a six month period the average precision attainable in the PIXE analysis was determined to be <math>1.6\%</math>. Using a calibration procedure, where matrix corrections from fundamental parameters were used to establish apparent thin-target sensitivities, the accuracy obtained was better than <math>3\%</math>. /Accepted for publication in Nucl. Instr. Meth. in Phys. Res. (1984)/</p> <p>Corrections for X-ray self-absorption and proton stopping power were performed on metal-doped plastic standards of intermediate thicknesses (<math>1.5 - 11 \text{ mg/cm}^2</math>). The accuracy of the results after corrections was better than <math>5\%</math> even for the thickest samples. /Nucl. Instr. Meth. <b>181</b> (1981) 179-183/</p> <p>In a set-up for proton microprobe analysis beam dimensions down to <math>4 \times 15 \mu\text{m}^2</math> were achieved by focusing with a quadrupole doublet. X-ray analysis was made on thin sections of dermatological material. By scanning over the cross-section of a hair in <math>3 \mu\text{m}</math> steps steep gradients were found for some trace elements. /Accepted for publication in Scanning Electron Microscopy (1983)/</p> <p>The proton microprobe was compared with an electron microprobe in the analysis of duplicate thin sections of human skin. The results from adjacent sections were highly reproducible for both methods, though small (<math>&lt;30\%</math>) systematic deviations between the results from the two methods were seen. /Accepted for publication in Nucl. Instr. Meth. in Phys. Res. (1984)/</p> |  |                                               |                     |
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Sölvegatan 14  
S-223 62 LUND, Sweden

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Date October 24, 1983

TILL EVY OCH JENS



RAPID ROCK ANALYSIS AND MICROPROBE  
SCANNING OF DERMATOLOGICAL MATERIAL  
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PROTON-INDUCED X-RAY AND GAMMA-RAY EMISSION

by

LARS-ERIC CARLSSON

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## SUMMARY

Introduction

The development of X-ray methods for elemental analysis started when characteristic X-rays were discovered in 1913. In the late 1940's X-ray methods, using X-ray tubes for excitation and crystal spectrometers for detection, began to spread as a technique for quantitative analysis of both major and trace elements. The fields of application and the diversification and refinement in instrumentation of X-ray methods has since then been continuously developed and modern X-ray techniques consists of several different approaches, which differ greatly in their ways of producing and detecting the characteristic X-rays.

In geological research and in the processes of prospecting and exploration large number of samples often have to be analysed for many elements before an interesting area can be fully evaluated. Thus it is important to be able to use rapid multi-elemental methods with high sensitivities. In mineral prospecting the indication of rare unexpected elements is important, as is the possibility of making quantitative analyses without time consuming sample preparation, e.g. direct analysis of drill cores. The demand for high accuracy and precision is especially important in geological research work since small changes in the concentrations of the major elements may also be of significance.

Another large field of application is the analysis of biological materials, where minor elements play important roles in the basic biological processes and trace metals are interesting both as essential elements and when they appear in toxic concentrations. Applications in this field often place high demands on both spatial resolution and elemental sensitivities.

One of the latest X-ray techniques employs accelerated protons or helium ions for the production of inner-shell vacancies and an energy dispersive solid state detector for the measurement of the characteristic X-rays. This method, called Particle-Induced X-ray Emission (PIXE) analysis was shown to be capable of multi-elemental determinations down to the  $10^{-12}$  gram level

in a thin sample by Johansson, Akselsson and Johansson in 1970 (1).

The use of highly collimated and focused particle beams became an important field of PIXE analysis in 1972, when Cookson et al. (2) carried out PIXE analysis with a proton beam diameter of three to four micrometres. With such a proton microprobe low elemental concentrations in fine structures can be measured and elemental maps with high spatial resolution may be obtained.

The PIXE technique is now in use at many laboratories with small or medium-sized accelerators all over the world, and three international conferences wholly devoted to PIXE analysis and its analytical applications have been held in Lund in 1976 (3), in Lund again in 1980 (4) and in Heidelberg in 1983 (5).

#### Properties of PIXE

The PIXE method has several properties, which together make it a unique tool in many analytical applications.

The truly multi-elemental behavior of PIXE allows for the determination of an amount or an upper limit for about 80 elements of the 92 existing in nature with one short irradiation. The lightest elements have X-ray energies too low to be measured.

The analysis is non-destructive, though the analytical conditions are often close to the levels where the effects of heating or radiation from the beam are visible.

Using beams of protons high excitation intensities are available and the level of the X-ray background is low. Thus, it is possible to detect elemental masses down to 0.1 ng in a 10  $\mu\text{g}$  sample deposited on a thin backing. In samples thicker than the proton range ( $>0.1$  mm) the concentration detection limits attainable are typically in the 0.5 to 50  $\mu\text{g/g}$  range depending on sample type.

The analysis times needed to reach these detection limits lie typically in range of one to five minutes, though for applications where ten times higher detection limits are

sufficient times below ten seconds can be used.

The sensitivity over the whole elemental range is a smooth and only slowly varying function of atomic number and can be described quite accurately by both theoretical and semi-empirical expressions. Thus, in many applications very simple calibration procedures can be used and no tedious element by element calibration has to be performed.

### Proton microprobe

Elemental analysis with high spatial resolution and detection limits down to about  $5 \mu\text{g/g}$  are unique features of the proton microprobe. Beam diameters between one and five micrometres have been obtained at several laboratories (6). The small proton beam is produced by first collimating the beam to about  $0.1 \times 0.1 \text{ mm}^2$  and then focusing with a magnetic or electrostatic lens system. In fig.1 the main features of a proton microprobe can be seen.

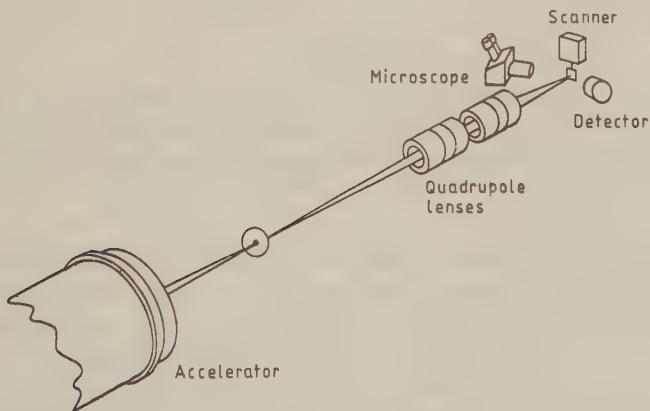


Figure 1. The principal components of a proton microprobe.

Topographical pictures of elemental concentrations in a sample can be determined by either mechanical scanning of the sample or by electrostatic or magnetic scanning of the proton beam.

There are several fields of application where the proton microprobe can find its use as an analytical tool, e.g. biology, mineralogy and materials analysis.



### Complementary methods

In its interaction with the nuclei of the sample atoms the proton beam can be scattered or induce nuclear reactions. These reactions may be used for the analysis of nuclides and are especially sensitive to the lighter elements, which cannot be detected by their X-rays. The detection of particles or gamma-rays from these processes can be carried out simultaneously with the X-ray measurements.

In thick samples the Proton-Induced Gamma-ray Emission (PIGE) analysis, where gamma-rays from nuclear de-excitation processes are measured in a solid-state detector, is favourable for the analysis of elements with atomic numbers 3,5,9,11,12 and 13. In thin samples protons scattered in forward and backward angles from the beam can be measured and from the proton energy spectra, in principle all of the light elements may be determined.

### Thesis

The aim of this work has been, firstly, to develop and apply the thick-target PIXE technique to the analysis of geological material and, secondly, to create a set-up for proton microprobe analysis.

The work on geological samples was started in 1978 with an investigation of the capabilities of X-ray and nuclear techniques in scanning drill core analysis (7). This investigation was initiated as a project in cooperation with the prospecting department of Boliden Mineral AB, Boliden. It was shown that the PIXE technique with the aid of simultaneous PIGE analysis, could give sufficiently low detection limits for all elements but gold and the platinum metals and that the errors in the analysis due to the heterogeneity of the drill core matrix were relatively low (see paper II). The PIXE-PIGE technique could also be shown to be capable of rapid analysis with high accuracy of practically all major and many trace elements in pellets of silica-based rock material (see papers I and III).

The work on the production of a proton microbeam with resolution down below the ten micrometre level was started in 1978 with the installation of a quadruplet lens system and target chamber made by Scanditronix AB in Uppsala. The system was kindly lent to us by the Gustaf Werner Institute, University of Uppsala. After many experiments with different approaches to the experimental parameters the system in 1982 was capable of producing a beam spot with a minimum width of four micrometres. The use of this microprobe in dermatological research is demonstrated in papers IV and V.

### Paper I

The simultaneous use of X-ray and gamma-ray detection from the irradiation of geological material with 2.5 MeV protons has been applied to the analysis of silica-based rock material. The elements with atomic numbers 3,5,9 and 11 to 13 are detectable by gamma-ray analysis and all heavier elements by X-ray analysis. In the analysis of geological material it is important to make accurate analysis of all major elements in the sample as well as determining as many minor and trace elements as possible. The PIXE analysis could be easily applied to this problem since major elements usually have low X-ray energies which can prevented from reaching the X-ray detector by the insertion of a filter. With a very small hole in the filter the signals from the lighter elements can be kept at a level suitable for analysis. With such an arrangement much higher beam intensity can be used which increases the sensitivity in the analysis of heavier elements and the elements determined from gamma-ray analysis.

### Paper II

The use of the PIXE method for drill core analysis in an atmospheric environment was investigated. The analysis of drill cores is an important tool in mineral prospecting and a routine method for automatic elemental scanning could provide significant improvements to the process of prospecting. A set-up for proton beam irradiation in air was designed so that the number of protons hitting the sample could be monitored by measuring the number of protons back-scattered in the exit

window with a surface barrier detector.

The PIXE technique was compared with secondary-target mode X-ray fluorescence (XRF) analysis and it was found that both systems give similar detection limits. The PIXE technique, though, has the advantage of simultaneous light element analysis from the gamma-ray detection also with the samples in air. The errors introduced by performing matrix corrections on heterogeneous samples were calculated for both systems and here the PIXE method was shown to be much less sensitive to variations in the matrix.

### Paper III

By repeated analyses of international geological standards during a six-month period the average reproducibility of the results of the PIXE analysis was determined to be 1.6 % (one S.D.). For each of the standards the concentration sensitivity (pulses/ $\mu\text{C}$  per  $\mu\text{g/g}$ ) was determined from the absolute X-ray yield and the given reference values. Matrix corrections calculated from fundamental parameters were applied to these sensitivities, thus forming apparent thin-target yields (pulses/ $\mu\text{C}$  per  $\mu\text{g/cm}^2$ ), which have built into them corrections for systematic errors in the thick-target matrix calculations, and are, in principle, only valid for the analysis of samples with a matrix similar to the ones of the standard samples used. The accuracy of the analysis of silica-based rock material using this calibration was determined to be better than 3 %.

### Paper IV

Corrections for X-ray self-absorption and proton stopping power were performed on samples with one to four layers of metal-doped polymer foils with total thicknesses ranging from 1.5 to 11  $\text{mg/cm}^2$ . These matrix corrections were based on fundamental parameters with the sample thicknesses determined from X-ray transmission measurements. The accuracy of the results after the matrix corrections was better than about 5 % even for the thickest layers, which implies that the errors in matrix corrections for samples with a light element matrix are lower than about 5 %.

Paper V

The elemental content of dermatological material has been determined *in situ* with high spatial resolution by scanning with a proton microprobe. The samples were thin (about  $0.5 \text{ mg/cm}^2$ ) frozen hydrated or freeze-dried sections obtained after freeze-quenching. A hair sample was scanned with a proton beam size of  $4 \times 15 \text{ } \mu\text{m}^2$  and a sample of human skin was analysed with a  $10 \times 100 \text{ } \mu\text{m}^2$  beam. In the hair section steep gradients of some trace elements were found.

The analysis of trace metal variations along a hair strand is also a suitable task for the PIXE method. Results obtained earlier from longitudinal scanning along the hair with a millimetre beam was presented. In this analysis the sample was kept in a helium atmosphere to diminish the heating in the hair.

Paper VI

The proton microprobe (PMP) was compared with the electron microprobe (EMP) in the analysis of duplicate thin sections of human skin. The results from adjacent sections were highly reproducible (typically better than 20 %) for both methods.

The quantitative procedure with the PIXE method uses general semi-empirical sensitivities verified by a thin-target calibration. The samples are transparent to the proton beam and thicknesses needed in the quantitative process were determined by using the yield of bremsstrahlung from the sample. In the electron microprobe the samples stop the electron beam and concentrations are determined by the use of peak to background values from a standard sample. The results from the PMP werer systematically a little lower than the EMP values, though the differences were typically smaller than 30 %.

The higher sensitivity obtained with the PIXE analysis revealed interesting gradients of calcium, iron and zinc on the borders between different strata of the skin. Though the EMP has a superior resolution for very thin samples the proton microprobe

can be considered to be a reliable and powerful complement to the existing tools of dermatological research.

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## RAPID DETERMINATION OF MAJOR AND TRACE ELEMENTS IN GEOLOGICAL MATERIAL WITH PROTON-INDUCED X-RAY AND GAMMA-RAY EMISSION

Lars-Eric CARLSSON and K. Roland AKSELSSON

*Department of Nuclear Physics, Lund Institute of Technology, Sölvegatan 14, S-223 62 Lund, Sweden*

The simultaneous detection of proton-induced X-rays and gamma-rays enables rapid determination of both major and trace elements in thick homogeneous samples. Using protons of energy 2.55 MeV, the atomic numbers of the elements detectable with this technique were  $Z = 3, 5, 9, 11-13$  from gamma-ray analysis and  $Z = 13-92$  from X-ray analysis. Thick target concentrations were determined from a thoroughly made thin target calibration and from matrix-dependent conversion factors calculated from fundamental parameters. The major compositions of samples needed in these calculations are derived in an iterative procedure using the spectra recorded. The method proposed has been tested on six USGS rock standards. Detection limits for one of the standards are presented. Approximately 20 elements were detected in each sample in 5 min irradiations. For elements well above their detection limits, the results are typically accurate to within 5%. The precision of repeated day-to-day analysis is about 2% (one standard deviation).

### 1. Introduction

Fast, reliable, multielemental analysis of geological material is a task of great importance in geological research and mineral exploration.

With the availability of high resolution Si(Li) detectors around 1970, the first reports of trace element analyses with particle-induced X-ray emission, PIXE, were published [1,2]. Since then, PIXE has proven to be a valuable analytical tool for routine analysis of a wide variety of sample types. The use of complementary methods, e.g., particle scattering analysis [3] or prompt gamma-ray analysis [4-6] simultaneously with PIXE analysis, further extends its capabilities.

In this study the simultaneous use of prompt gamma-ray analysis and PIXE analysis has been evaluated for thick homogeneous pellets of geological material. This combination was found to be effective and accurate. Special attention has been paid to the design of a proper X-ray absorber between the sample and the detector and to the accuracy of matrix corrections in the PIXE analysis.

### 2. Quantitative analysis of thick samples with PIXE

At most PIXE laboratories, accurate thin sample efficiency calibrations have been performed. It is then convenient to use these calibrations also for thick

samples by applying a thick target factor,  $f$ , to convert a fictive thin sample result (e.g., in  $\text{ng}/\text{cm}^2$ ) to a concentration (e.g., in ppm). The thick target factor,  $f$ , depends on the X-ray and proton energies, the matrix composition of the sample and the angles between beam, detector and the normal to the sample. The factor,  $f$ , is given by

$$f = Y_{\text{thick}}/Y_{\text{thin}} = \int_0^{E_0} T(x) \sigma(E) S^{-1} dE/\sigma(E_0), \quad (1)$$

where  $Y_{\text{thick}}$  is the thick sample yield of characteristic X-rays per  $\mu\text{C}$  and ppm,  $Y_{\text{thin}}$  the corresponding thin sample yield per  $\mu\text{C}$  and  $\text{ng}/\text{cm}^2$ ,  $\sigma(E)$  the ionization cross section at proton energy  $E$ ,  $E_0$  the proton energy at the sample surface (keV),  $x$  the pathlength in the sample of X-rays on their way towards the detector,  $T(x)$  the transmission of X-rays out of the sample and  $S$  the proton stopping power ( $\text{keV}/(\text{mg}/\text{cm}^2)$ ).  $S$  is a function of the matrix composition and the proton energy.

Fig. 1 shows the calculated conversion factors for US Geological Survey rock standard AGV-1. The procedure used in the calculations has been described by several authors [7,8]. We have used the polynomial fit given by Akselsson and Johansson [9] for X-ray ionization cross sections, stopping powers from Northcliffe and Schilling [10] and X-ray attenuation coefficients from Veigele [11].

In a similar way, factors which transform thin sample relative intensities of an element into thick sam-

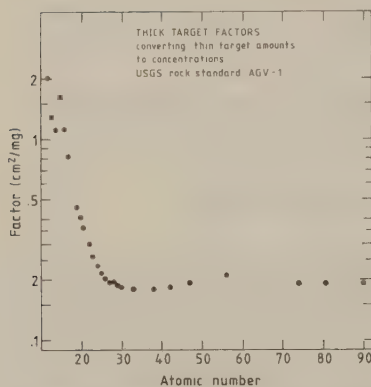


Fig. 1. Calculated thick target factors for the AGV-1 sample. The proton energy is 2.55 MeV and the detector is positioned at  $135^\circ$  to the beam

ple relative intensities has been derived. For elements heavier than iron, these factors are more or less independent of the matrix composition.

In all these calculations, the matrix composition of the sample has to be known. This fact constitutes a problem in many applications, where the composition of the light element fraction has to be guessed. By designing the analysing system as described in this work, practically all major elements are detected simultaneously and the total sample composition may be calculated by an iterative procedure.

The sensitivity of the thick target factor,  $f$ , to changes in the matrix composition can be seen in fig. 2, which shows the relative change of the correction factors due to a 10% decrease in the concentra-

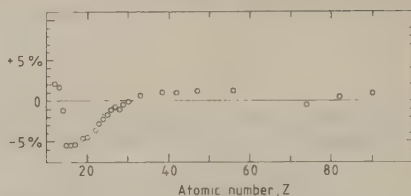


Fig. 2. Errors in thick target factors assuming a 10% too low value of the detected elements and consequently a too high value of oxygen.

tions of all the elements with  $Z$  larger than 10 and a corresponding increase in the oxygen content.

### 3. Quantitative gamma-ray analysis

The thick target yields of prompt gamma-rays from a specific proton-induced reaction are preferably determined by using calibration samples made of simple compounds. The yield,  $Y_{\text{cal}}$ , for a certain isotope in such a calibration cannot be used directly on other samples with different matrix compositions, since changes in stopping powers give changes in the mass per unit area penetrated by the beam. The yield from a sample is given by

$$Y_s = Y_{\text{cal}} \int_0^{E_0} \sigma(E)/S_s(E) dE / \int_0^{E_0} \sigma(E)/S_{\text{cal}}(E) dE, \quad (2)$$

where  $E_0$  is the initial proton energy,  $\sigma(E)$  the cross section for the reaction used at the proton energy  $E$  and  $S_s(E)$  and  $S_{\text{cal}}(E)$  the stopping powers of the unknown sample and the calibration sample respectively. Since the ratio  $S_s(E)/S_{\text{cal}}(E)$  is almost independent of  $E$ , it is possible to write  $S_s(E) =$

Table 1  
Ratio of stopping powers for C, SiO<sub>2</sub> and AGV-1 to Li<sub>2</sub>B<sub>4</sub>O<sub>7</sub> and NaCl

| Proton energy<br>(MeV)                    | Ratios to Li <sub>2</sub> B <sub>4</sub> O <sub>7</sub> for |                  |       | Ratios to NaCl for |                  |       |
|-------------------------------------------|-------------------------------------------------------------|------------------|-------|--------------------|------------------|-------|
|                                           | C                                                           | SiO <sub>2</sub> | AGV-1 | C                  | SiO <sub>2</sub> | AGV-1 |
| 1.0                                       | 1.10                                                        | 0.87             | 0.84  | 1.47               | 1.16             | 1.13  |
| 1.5                                       | 1.06                                                        | 0.91             | 0.88  | 1.35               | 1.16             | 1.12  |
| 2.0                                       | 1.07                                                        | 0.93             | 0.90  | 1.31               | 1.14             | 1.11  |
| 2.5                                       | 1.07                                                        | 0.94             | 0.92  | 1.29               | 1.13             | 1.11  |
| Maximum error<br>introduced by<br>eq. (3) | 4%                                                          | 4%               | 4%    | 9%                 | 3%               | 1%    |

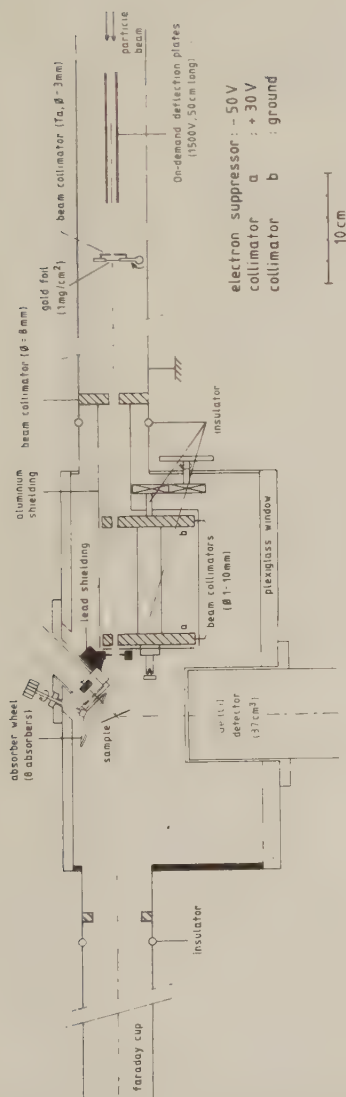


Fig. 3. The PIXIE setup in Lund.

$S_s(E_1) h(E)$  and  $S_{cal}(E) = S_{cal}(E_1) h(E)$ , which means that relation (2) may be approximated by

$$Y_s = Y_{cal} S_{cal}(E_1) / S_s(E_1). \quad (3)$$

For protons with initial energy  $E_0 = 2.55$  MeV, we have used  $E_1 = 1.5$  MeV. Table 1 shows the ratio  $S_s(E)/S_{cal}(E)$  for different  $E$  and a few different matrices and calibration standards.

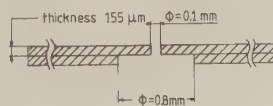
Since the ratio  $S_s(E)/S_{cal}(E)$  is a smooth slowly varying function of the proton energy,  $E$ , the maximum error introduced by using formula (3) can be estimated as in table 1. Below 1.0 MeV cross sections,  $(E)$ , relevant in this context, are generally small compared to cross sections in the interval  $1.0 < E < 2.55$  MeV.

#### 4. Experimental

Samples of six different USGS rock standards were prepared by pressing approximately 2 g of powder to a 32 mm diameter pellet in a 10 ton hydraulic press. The samples were irradiated in vacuum with 2.55 MeV protons from the 3 MV tandem accelerator in Lund. The experimental chamber is shown in fig. 3. It is designed to permit accurate proton current integration for both thin and thick samples.

Samples of geological material are often electrically insulating and charge may easily build up to voltages over 10 kV during proton bombardment. This effect is avoided by spraying the samples with electrons from a heated carbon filament [12]. Reproducible and uniform beam intensity distribution on the sample was achieved by diffusing the beam through a 1 mg/cm<sup>2</sup> gold foil.

The signals from the 80 mm<sup>2</sup> (Si(Li) detector and the 37 cm<sup>3</sup> Ge(Li) detector were fed into separate ACDs connected to a Nuclear Data 6600 computer. The area of peaks in the gamma spectra were automatically integrated with a peak search routine in this computer, while the X-ray spectra were stored on



2-LAYER AL HOLE-ABSORBER

Fig. 4. External absorber designed for analysis of geological material.



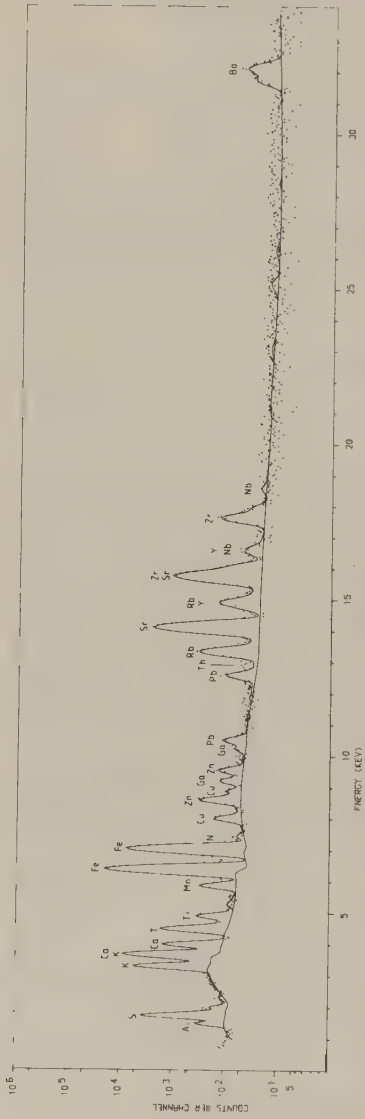


Fig. 5. X-ray spectrum of the AGV-1 sample. The irradiation time was 6 min and the count rate 1100 cps. The solid lines are computer fits as given by the HEX programme. The two-layer hole absorber (see Fig. 4) was used.

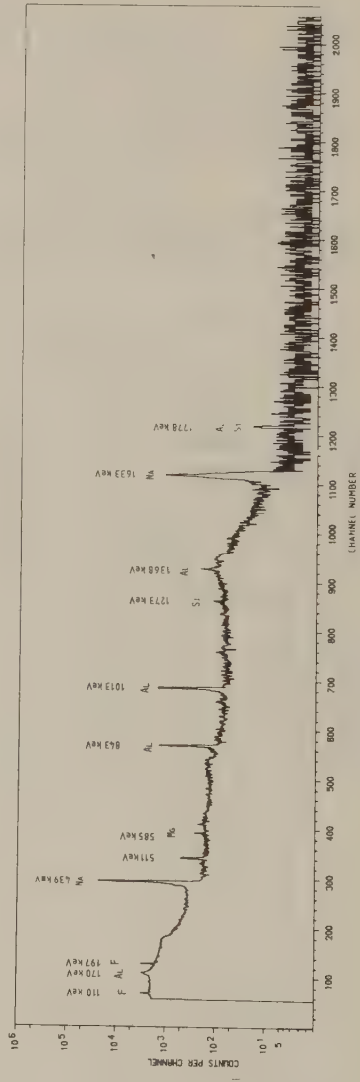


Fig. 6. Gamma-ray spectrum of the AGV-1 sample. The irradiation time was 3 min and the count rate 2500 cps.

magnetic tape for subsequent analysis with the HEX programme [13] on a UNIVAC 1100 computer.

By using an electrostatic on-demand beam deflection system for the X-ray detector system [14], pulse pile-up was kept low and no explicit deadline corrections had to be made. Small corrections (<7%) for the deadline in the ADC connected to the Ge(Li) detector were made.

A wheel in front of the X-ray detector holds seven different external absorbers. One of them (seen in fig. 4) is designed for the analysis of geological material. This absorber reduces the intensities of the elements from aluminium to manganese by factors of 2000 to 1200 and from iron by a factor of 500 (approximate values). The strong reduction of X-ray intensities from the most abundant elements enables the use of high beam currents and gives increased sensitivity for heavy elements and for the elements determined by gamma-ray analysis.

By making this absorber of aluminium and using a two-layer design, the diameter of the small hole can be of the same order as the thickness. A corresponding absorber made of organic material would of course require much thicker layers which would give rise to an increased sensitivity to small deviations in the angle between the normal of the absorber and the sample-detector direction. For small-hole sizes edge effects result in a decreasing effective hole area with decreasing X-ray energy. In our geometry, the effective area of the minor hole was found to be 40% lower for Si  $K_{\alpha}$  X-rays than for Mn  $K_{\alpha}$  X-rays. No fluorescence in the absorber producing Al K X-rays has been seen.

Fig. 5 shows a X-ray spectrum acquired with this absorber. The solid lines are the fits of peaks and background as given by the HEX programme.

Relative X-ray intensities corresponding to an assumed standard matrix composition have been used for the computer fit to the spectra; preliminary concentrations corresponding to this matrix were also determined. In an iterative procedure calculated concentrations are corrected and form a new matrix composition with oxygen filling up to 100%. For the USGS samples, the values were sufficiently close after one or two iterations and no iterations of the relative intensities were needed.

With the Ge(Li) detector positioned as shown in fig. 3, the gamma-rays have to penetrate through the sample to reach the detector. The pellets of USGS standards were made several years ago and had a backing of organic material 6–8 mm thick. The

Table 2

Nuclear reactions and corresponding relative yields used in the gamma-ray analysis

| Reaction                                         | Gamma-ray energy (keV) | Calibration sample                | Relative yield   |
|--------------------------------------------------|------------------------|-----------------------------------|------------------|
| ${}^7\text{Li}(p, p' \gamma){}^7\text{Li}$       | 478                    | $\text{Li}_2\text{B}_4\text{O}_7$ | 300              |
| ${}^{10}\text{B}(p, \alpha \gamma){}^7\text{Be}$ | 431                    | $\text{Li}_2\text{B}_4\text{O}_7$ | 45 <sup>a)</sup> |
| ${}^{19}\text{F}(p, p' \gamma){}^{19}\text{F}$   | 197                    | GSP-1 <sup>b)</sup>               | 460              |
| ${}^{23}\text{Na}(p, p' \gamma){}^{23}\text{Na}$ | 439                    | G-2 <sup>b)</sup>                 | 100              |
| ${}^{25}\text{Mg}(p, p' \gamma){}^{25}\text{Mg}$ | 585                    | DTS-1 <sup>b)</sup>               | 0.15             |
| ${}^{27}\text{Al}(p, p' \gamma){}^{27}\text{Al}$ | 1013                   | GSP-1 <sup>b)</sup>               | 3.2              |

<sup>a)</sup> This value has not been corrected for interference from the  ${}^7\text{Li}(p, n \gamma){}^7\text{Be}$  reaction.

<sup>b)</sup> USGS standards.

transmission of gamma-rays through this layer was calculated and differences were corrected for. The corrections were from 3 to 8% with an uncertainty of less than 1%. In the calibration of gamma-ray yields, the USGS standard having the lowest statistical error for each element was used except for lithium and boron, where a pellet of  $\text{Li}_2\text{B}_4\text{O}_7$  with the same backing as the rock standards was used. Table 2 shows the reactions used and the corresponding gamma-ray energies. The calibration sample used for each reaction is also stated. The gamma-ray yields are normalized to sodium and are not corrected for detector efficiency. A spectrum acquired during a 3 min irradiation is shown in fig. 6.

## 5. Results and discussion

The concentrations determined for the AGV-1 sample (fig. 5) are listed in table 3. The statistical uncertainties are expressed as one standard deviation. The ratios of the determined concentrations to the concentrations given by Flanagan [15] have been calculated for the six samples. The averages and the standard deviations of the ratios are also found in table 3.

If there was a systematic error in proton stopping power data, this would show up as a deviation from unity in the average ratios of the heavy elements and in the same way the values of the light elements would be most affected by a systematic error in the X-ray attenuation coefficients. No such systematic deviation is present in table 3, indicating that possible errors in the data used are too small to have any sig-

Table 3

Results from the analyses of six USGS rock standards <sup>a)</sup> by proton induced reactions. Elements determined by gamma-ray analysis are in the upper part of table.

| Element | AGV-1               |                             |                                     | Average of ratios <sup>b)</sup> | Standard deviation of ratios <sup>b)</sup> |
|---------|---------------------|-----------------------------|-------------------------------------|---------------------------------|--------------------------------------------|
|         | Concentration (ppm) | Statistical uncertainty (%) | Ratio to value reported by Flanagan |                                 |                                            |
| Li      | <25                 |                             |                                     |                                 |                                            |
| F       | 390                 | 8                           | 0.89                                | 0.85                            | 0.11                                       |
| Na      | 34 100              | 1                           | 1.07                                | 1.01                            | 0.04                                       |
| Mg      | 9500                | 30                          | 1.13                                |                                 |                                            |
| Al      | 102 000             | 2                           | 1.12                                | 1.08                            | 0.11                                       |
| Al      | 84 100              | 9                           | 0.92                                | 0.95                            | 0.06                                       |
| Si      | 264 000             | 2                           | 0.96                                | 0.96                            | 0.05                                       |
| K       | 27 100              | 2                           | 1.13                                | 1.18                            | 0.09                                       |
| Ca      | 36 700              | 2                           | 1.05                                | 1.05                            | 0.04                                       |
| Ti      | 6580                | 3                           | 1.06                                | 1.07                            | 0.04                                       |
| Mn      | 832                 | 5                           | 1.09                                | 1.07                            | 0.08                                       |
| Fe      | 36 700              | 1                           | 0.97                                | 0.95                            | 0.04                                       |
| Ni      | 12                  | 41                          | 0.67                                |                                 |                                            |
| Cu      | 55                  | 9                           | 0.92                                |                                 |                                            |
| Zn      | 81                  | 4                           | 0.96                                | 1.00                            | 0.06                                       |
| Ga      | 19                  | 10                          | 0.93                                | 0.93                            | 0.11                                       |
| Rb      | 74                  | 3                           | 1.10                                | 1.11                            | 0.11                                       |
| Sr      | 675                 | 2                           | 1.03                                | 1.03                            | 0.05                                       |
| Y       | 17                  | 13                          | 0.79                                |                                 |                                            |
| Zr      | 229                 | 3                           | 1.02                                | 0.92                            | 0.07                                       |
| Nb      | 10                  | 16                          | 0.67                                |                                 |                                            |
| Ba      | 1190                | 6                           | 0.99                                | 1.00                            | 0.09                                       |
| Pb      | 42                  | 5                           | 1.19                                | 1.08                            | 0.14                                       |
| Th      | 5                   | 45                          | 0.78                                |                                 |                                            |

<sup>a)</sup> AGV-1, G-2, GSP-1, BCR-1, W-1 and DTS-1.

<sup>b)</sup> 4–6 samples.

nificant impact on the results. It is still possible, however, that a small systematic increase or decrease in both stopping power data and X-ray attenuation data is compensated for by a small error in the level of the thin target calibration.

Detection limits for the AGV-1 sample with a 5 min irradiation time are shown in fig. 7. The detection limit is defined as  $3\sqrt{B}$ , where  $B$  is the background in an energy interval equal to 2 fwhm around the peak energy.

The detection limit for X-ray energies over about 12 keV is strongly influenced by the background from Compton scattered gamma-rays. The level of this background is determined mainly by fluorine and sodium abundant at the 0.1% and 2% levels respectively.

The results of the X-ray analysis agree typically

to within 5% with the values given by Flanagan, implying the accuracy of our routines to be better than about 5%. The precision of day-to-day analyses is around 2% (one standard deviation). The mean value of the standard deviations in table 3 is 7%. This variation can be explained by heterogeneities at the mg-level (the total mass analyzed with PIXE is about 5 mg) and by particle size effects, which influence all elements in the PIXE analysis. More than 90% of the X-rays from heavier elements originate from a layer approximately 30  $\mu\text{m}$  thick.

The quoted accuracy is valid for a variety of sample compositions. The silver concentration in a pure silver sample was determined. This sample is used as a general standard at our laboratory and the standard deviation of approximately 500 analyses during the last two years was 2.5% [16]. The mean

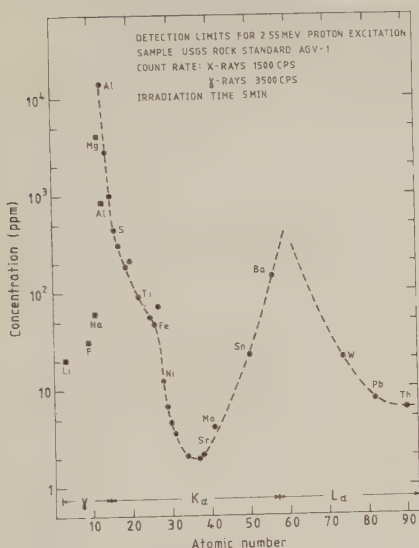


Fig. 7. Detection limits for the AGV-1 sample with a 5 min irradiation time, 1500 cps in the Si(Li) detector and 3500 cps in the Ge(Li) detector. The two-layer hole absorber was inserted in front of the X-ray detector. The circular dots are detection limits from the X-ray analysis and filled squares those from the gamma-ray analysis.

value of the silver concentration obtained was 97%. A single analysis of a pure  $\text{Fe}_2\text{O}_3$  sample (70% Fe) gave an iron concentration of 68%.

The precision in the gamma-ray analysis simultaneously with the PIXE analysis is estimated to be about the same as in the X-ray analysis. Only one calibration sample has been used per element (see table 2) and the accuracy is better than about 10%.

## 6. Conclusions

The use of proton-induced gamma-ray analysis simultaneously with PIXE analysis extends the number of elements which can be determined, and

increases the accuracy of the matrix corrections in the PIXE analysis.

The analyses of accurately known rock standard samples from the US Geological Survey indicate that in PIXE analysis the matrix correction factors achieved with the fundamental parameters have errors smaller than about 5% and confirm the validity of the thin-target calibration of the PIXE system in Lund.

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APPLICABILITY OF PIXE AND XRF TO FAST DRILL CORE  
ANALYSIS IN AIR

Lars-Eric Carlsson and K. Roland Akselsson

Department of Nuclear Physics, Lund Institute of Technology  
Sölvegatan 14  
S-223 62 LUND, Sweden

Abstract

The properties of particle-induced X-ray emission, PIXE, and secondary target mode X-ray fluorescence, XRF, applied to the analysis of unprepared drill cores in open air have been evaluated. Typical detection limits for elements heavier than Mg have been determined for a PIXE-system with an external 2.55 MeV proton beam and for an XRF-system with Ti, Mo and Tb secondary targets. These two systems were found to have similar detection limits for most elements in a typical geological sample. The heterogeneous composition of drill cores prevents the performance of accurate matrix corrections, though calculations using fundamental parameters show that in the PIXE analysis of elements heavier than Ca, these corrections are much less sensitive to variations in the matrix composition than in the XRF analysis.

Introduction

A large mineral company in Sweden stores 30 - 40 km of drill cores in a central storage every year. These cores are visually inspected for their mineral composition and only small fractions are prepared for accurate elemental analyses. A technique for fast analyses of all metals of prospecting interest without time consuming sample preparation would open new possibilities for successful prospecting efforts.

There are several reasons why energy-dispersive X-ray methods are suitable for such scanning drill core analysis. They are truly multi-elemental, giving signals from unexpected elements without any extra effort, which is an important property in elemental analysis

for prospecting purposes. Further, these methods are fast. With irradiation times in the range of 1-3 minutes it is possible to determine both major and trace elements below the 10 ppm level. X-ray elemental analysis techniques are nondestructive and for some applications little or no sample preparation is necessary.

Accurate analyses of undiluted pressed pellet samples in vacuum have earlier demonstrated with both PIXE and XRF using the fundamental parameter technique. Verbeke and Adams (1) used Ti and Mo secondary targets to determine elements heavier than Na in seven rock standards and stated an accuracy of approximately 10%. We have earlier used 2.55 MeV protons to determine Li, F and all elements heavier than neon in six rock standards by simultaneous X-ray and gamma-ray analysis (2). Accuracies typically better than 5% and 10% respectively were achieved.

In this work the main purpose has been to establish comparable detection limits for PIXE and XRF analysis and to evaluate the accuracy of matrix correction for these two modes of X-ray excitation.

### Experimental

A homogeneous pellet of geological material was irradiated with 2.55 MeV protons, which emerged out of the accelerator vacuum into air through a 7  $\mu\text{m}$  thick polyamid foil (Kapton). In fig.1 the principal view of this external beam set-up is showed.

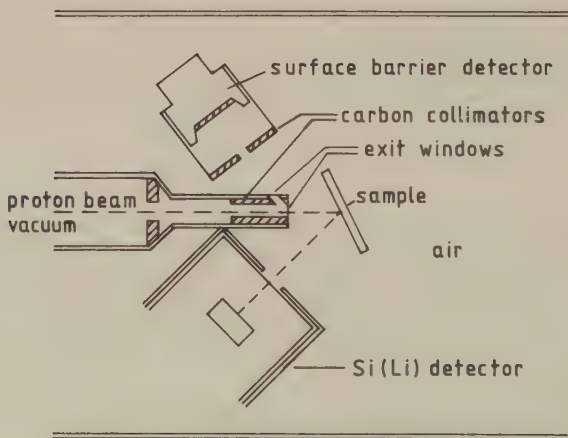


Fig. 1. A schematic view of the set-up for external beam PIXE analysis.

## APPLICABILITY OF PIXE AND XRF

A 25 mm path of air between sample and detector window renders X-ray analysis of Na and Mg impossible. Beam current on the sample is monitored by measuring protons backscattered from the exit foil. In front of the Si(Li) detector a 0.3 mm thick Al absorber with a small hole was placed to reduce the intensity of X-rays from the abundant light elements in the sample.

The sample analysed with external beam PIXE was also analysed by XRF, with a Mo secondary target at the XRF laboratory in Gothenburg (3). This set-up is designed with a three axis geometry to make use of the polarization effects of primary bremsstrahlung in secondary target and sample. By careful collimation of beam paths and the detector crystal, the background on the low energy side of the scattered exciting radiation is very low. This analysis was also performed in air.

### Matrix effects in PIXE and XRF analysis

The efficiency calibrations of XRF and PIXE systems are often performed using thin target standards, thus being independent of matrix effects. Systems calibrated in this way can be used in combination with the fundamental parameter technique to do accurate analysis of thick homogeneous samples. Energy dispersive systems may easily be designed to measure practically all major elements simultaneously and no preknowledge of the sample to be analysed is necessary. In this way the sample composition can be determined by iterative calculations of matrix correction factors, which convert the thin target yield (e.g. pulses per  $\text{ng}/\text{cm}^2$ ) to a thick target yield (e.g. pulses per ppm). These correction factors are composed of one component caused by effects on incoming exciting radiation -  
 - slowing down of particles (PIXE) and absorption of X-rays (XRF) -  
 - and of one component caused by absorption of induced characteristic X-rays. Here two major differences can be found between PIXE and XRF:

- 1) The component due to the exciting radiation is for PIXE less dependent on matrix composition and generally this component works in a compensating manner to the absorption of the induced X-rays. This is generally not the case for XRF.
- 2) For XRF with Mo and Tb excitation a thicker layer is analysed giving stronger corrections for the absorption of the induced X-rays.

This advantageous matrix dependence of PIXE is especially important in the analyses of heterogeneous samples, where only approximate average concentrations can be calculated from the recorded spectrum. An element detected could have originated from a matrix quite different from this average composition. Fig. 2 shows what the error would be if matrix corrections were made for a pure quartz matrix when the composition really was half quartz and half iron pyrite or half quartz and half carbon. For PIXE analysis the X-ray self absorption and the proton stopping power compensate each other

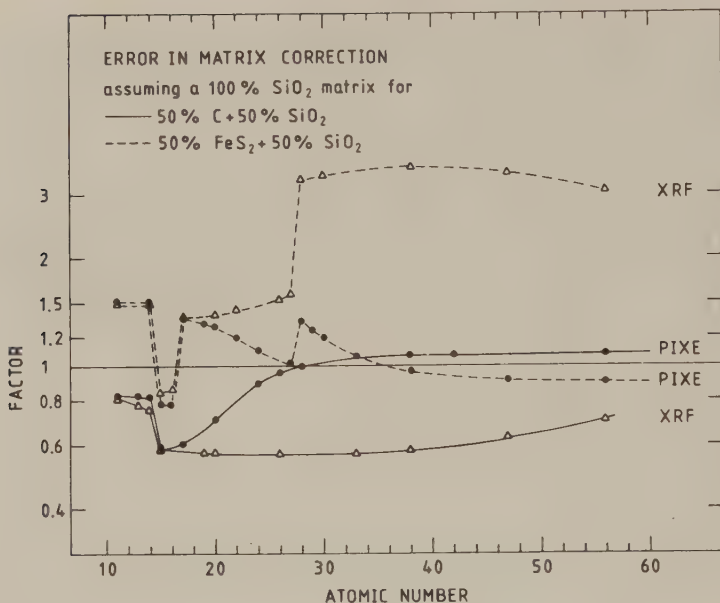


Fig. 2. Error in matrix correction factors due to the assumption of a pure quartz matrix. Filled circles are for PIXE and open triangles are for XRF.

around  $Z = 30$  making matrix correction for those elements quite independent of the matrix composition.

### Results

The spectrum of a homogeneous pellet of a typical geological material analysed with external beam PIXE is shown in fig. 3. The elemental composition determined is found in table I. From this spectrum detection limits corresponding to an irradiation time of 3 minutes and a count rate of 1500 pulses per second have been calculated. The detection limit was defined as  $3\sqrt{B}$ , where  $B$  is the background in a 2 FWHM region under the peak of interest. The characteristic peaks were not included in this background. An XRF system needs typically three different excitation modes to cover the same elemental range as PIXE. The detection limits for XRF have been calculated for three excitations using an irradiation time of 1 minute and a count rate of 2000 pulses per second each. The higher count rate for the XRF analyses is used because the pulse pile-up peaks from the scattered exciting radiation do not fall in the

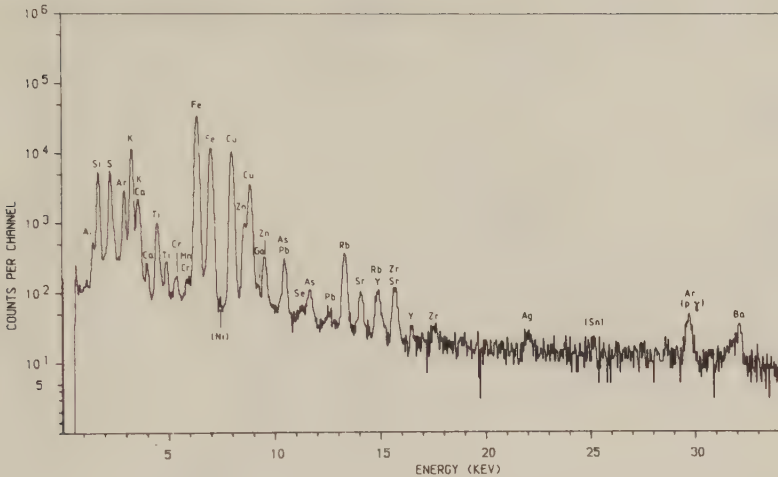


Fig. 3. A spectrum of a homogeneous pellet of a typical geological material analysed in air with PIXE. The proton energy at the sample surface was 2.55 MeV. The total number of pulses in the spectrum is 500,000.

Table I. Elemental concentrations and relative uncertainty (%) for the typical geological sample used.

|    |         |      |    |         |      |
|----|---------|------|----|---------|------|
| Al | 5.6 %   | (10) | Ga | 9 ppm   | (17) |
| Si | 22 %    | (6)  | As | 50 ppm  | (7)  |
| S  | 6.4 %   | (5)  | Se | 3 ppm   | (30) |
| K  | 3.9 %   | (5)  | Rb | 64 ppm  | (6)  |
| Ca | 0.16 %  | (6)  | Sr | 17 ppm  | (8)  |
| Ti | 0.17 %  | (6)  | Y  | 4 ppm   | (25) |
| Cr | 130 ppm | (13) | Zr | 41 ppm  | (6)  |
| Mn | 100 ppm | (40) | Ag | 13 ppm  | (24) |
| Fe | 5.5 %   | (5)  | Ba | 320 ppm | (10) |
| Cu | 0.44 %  | (5)  | Pb | 11 ppm  | (11) |
| Zn | 310 ppm | (5)  |    |         |      |

X-ray region of interest. The spectrum from Mo excitation of the same sample as used in fig. 3 is seen in fig. 4. The detection limits for Mo excitation are calculated from this spectrum and from the concentrations given in table I. Detection limits for the Tb excitation have been evaluated from ref. (4). For both the proton and the Mo excitation the count rate was limited by the 5.5 % Fe

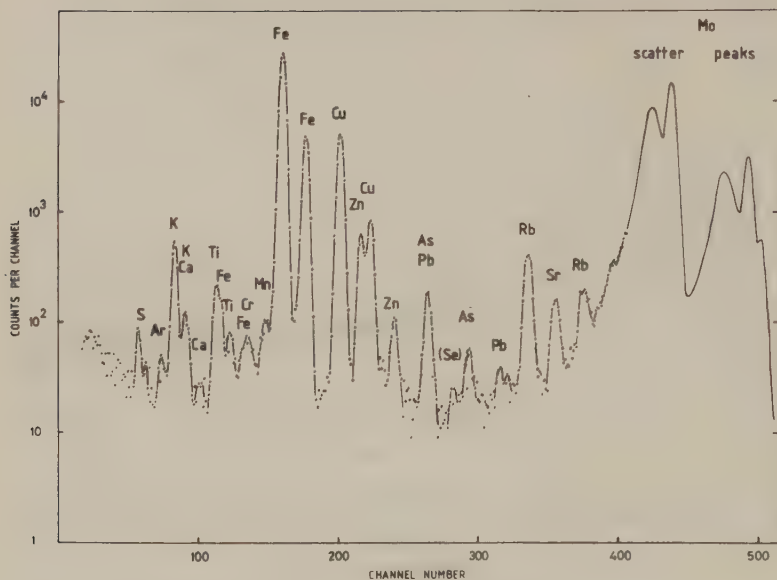


Fig. 4. A spectrum of the sample used in fig. 3 when analysed with Mo secondary target in air. The total number of pulses in the spectrum is 500,000.

content. In the case of Tb excitation the count rate practically always is dominated by the scattered radiation and here the detection limits will be fairly independent of the matrix composition.

The detection limits for 2.55 MeV proton, Tb and Mo excitations are given in fig. 5. Also included in fig. 5 is a coarse estimation of the detection limits using Ti excitation, which will be very dependent on Ca, K and S concentrations in the sample. Data from vacuum analyses in refs. (1) and (5) have been used in the estimation.

Also for the analysis of homogeneous pellets the advantageous behaviour of matrix correction in PIXE analysis is useful. If a systematic error were present in the determination of major elements, the iteratively calculated correction factors would introduce an additional error. This is illustrated in fig. 6, where the error in correction factors due to 10% low concentrations of all elements determined and a corresponding increase of the oxygen content for the sample listed in table I are plotted. Fig. 2 and fig. 6 show that PIXE is more suited to be used with the fundamental parameter technique than XRF.

In the case of pressed pellet samples XRF analysis of heavier elements is less sensitive than PIXE to particle size effects,



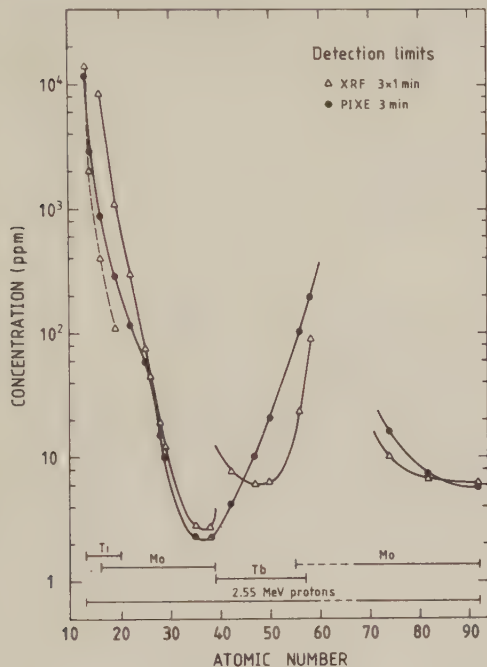


Fig. 5.

Detection limits for PIXE and XRF. Calculated for totally 3 minutes analysis of the sample used for the spectra in figures 3 and 4.

where a different composition of small particles at the surface of the pellet can make this layer non-representative to the bulk sample. In PIXE more than 90% of the X-rays from the heavier elements come from a layer of about 30  $\mu\text{m}$ , which means that particle size effects would influence the analysis of all elements.

Simultaneously with the accumulation of the PIXE spectrum it is possible to measure the gamma-rays from proton induced nuclear reactions in the sample. The gamma-ray spectrum in fig. 7 was recorded simultaneously with the spectrum in fig. 3 using the external proton beam. This simultaneous use of gamma-ray analysis makes it possible to determine Li, F, Na, Mg and Al simultaneously with the X-ray analysis (2). Detection limits for these elements using the same conditions as in fig. 5 are found in table II.

### Conclusions and discussions

Energy dispersive X-ray analysis using protons or monoenergetic X-rays for excitation are well suited for fast multi-elemental monitoring of drill cores. The advantageous matrix dependence of

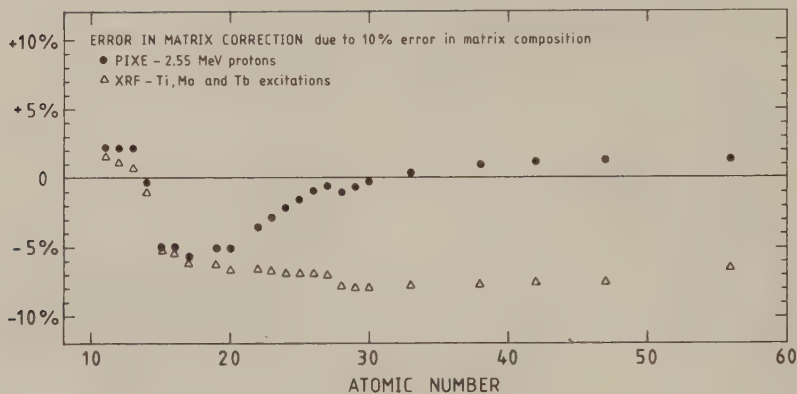


Fig. 6. Error in matrix correction due to the assumption of 10% too low values of all elements determined compensated by an increased oxygen content. The sample composition in table I was used.

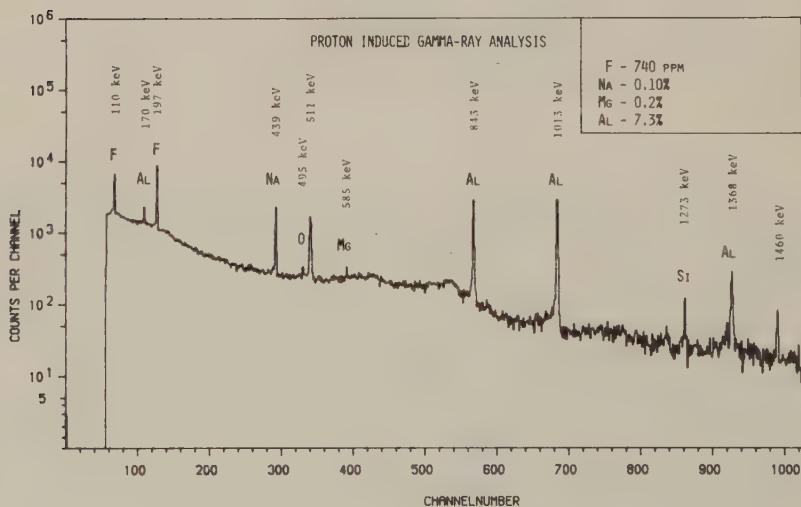


Fig. 7. Gamma-ray spectrum acquired with a Ge(Li)-detector simultaneously with the X-ray spectrum in fig. 3.

## APPLICABILITY OF PIXE AND XRF

Table II. Detection limits for the gamma-ray analysis.

| Element | Detection limit |
|---------|-----------------|
| Li      | 40 ppm          |
| F       | 50 ppm          |
| Na      | 100 ppm         |
| Mg      | 0.4 %           |
| Al      | 0.1 %           |

PIXE makes it possible to do rather accurate determinations of elements heavier than Ti even in heterogeneous samples. With the PIXE technique it is also possible to determine many elements lighter than Si by simultaneous gamma-ray analysis.

The depth of analysis for heavier elements is greater for XRF than for PIXE, which is an advantage when average concentrations in heterogeneous samples are wanted. Another advantage of XRF systems is the commercial availability and relatively low capital cost, though a PIXE system based on one of the small easy-to-handle tandem accelerators that are commercially available today, will have about the same operational cost as an XRF system.

#### Acknowledgements

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ACCURACY AND PRECISION IN THICK-TARGET PIXE-PIGE  
ANALYSIS DETERMINED WITH GEOLOGICAL STANDARDS.

Lars-Eric Carlsson

Department of Nuclear Physics, Lund Institute of Technology,  
Sölvegatan 14, S-223 62 LUND, Sweden

ABSTRACT

Simultaneous X-ray (PIXE) and gamma-ray (PIGE) analyses have been performed on international geological standards. Elements with atomic numbers 3,5,9,11,12 and 13 were determined from gamma-ray analysis and all heavier elements from X-ray analysis. To study the precision and accuracy attainable, one pellet for each of five international standards and one quartz standard was made. Each sample was analysed 10 to 19 times during a six-month period. The results were used for calibration in such a way that apparent thin-target sensitivities were established to give correct values after matrix corrections with the fundamental parameter technique. The mean of the precision, expressed as one standard deviation, was 1.6 % for elements with good pulse statistics. Ten duplicate pellets of a standard, made and thoroughly calibrated at the Department of Geology, Lund University, were analysed and the deviations from reference values for the elements Na, Al, Si, K, Ca, Fe, Rb and Sr were all less than 3.3 %.

INTRODUCTION

In an earlier paper both major and trace elements in six geological standards were analysed with Particle-Induced X-ray Emission (PIXE) and simultaneous Gamma-ray Emission (PIGE)<sup>1)</sup>.

Elements with atomic numbers 3,5,9 and 11 to 13 were determined from gamma-ray analysis and all heavier elements from X-ray analysis. The elemental concentrations were determined by applying thick-target factors to values obtained using thin-target sensitivities<sup>2)</sup>. The factors were calculated by integrating X-ray yield and self-absorption over the sample depth using fundamental parameters.

The results in the previous study were based on one single determination of each standard and for elements with low statistical uncertainties the average standard deviation of their ratios to reference values was 7 %. Reference values of elemental concentrations in geological standards are accurate to better than 1 to 2 %<sup>3)</sup> and thus these standards are suitable for a further evaluation of the precision and accuracy in thick-target PIXE-PIGE analysis.

#### MATERIALS AND METHODS

The samples were irradiated with a proton beam of 7 mm diameter and 2.55 MeV energy. Beam intensities from 50 to 100 nA were employed. The experimental set-up used has been described previously<sup>1,4)</sup>. The Ge(Li) detector had a resolution of 2.6 keV at 1274 keV and the Si(Li) detector 160 eV at 5.9 keV. The count rates for gamma rays and X-rays were kept below 10 000 and 2 000 pulses per second respectively. A program for the automatic integration of the six gamma-ray lines was implemented on a Nuclear Data 6620 computer. The net areas and statistical uncertainties were stored in 12 channels at the beginning of the X-ray spectrum. This X-ray spectrum was transferred to a Nord-500 computer where a fitting-program and a thick-target program were run. A heated carbon filament, which was used to prevent charge build-up in the samples, was later abandoned for a carbon foil ( $40 \mu\text{g}/\text{cm}^2$ ) placed about 3 cm in front of the sample.



In connection with a project where 400 pellets of geological material were analysed, one pellet for each of five international standards and one quartz standard was made. The standards came from the United States Geological Survey<sup>3)</sup> (AGV-1, GSP-1, G-2 and DTS-1) and from Zentrales Geologisches Institut, Berlin (GM) and were used to calibrate the sensitivities and to study the precision and accuracy of the analysis. The analyses were performed within 5 two-day periods stretching over 6 months. To further investigate the accuracy and to investigate the important question of sample homogeneity, ten duplicate samples of a standard SG, made and calibrated at the Department of Geology, Lund University, were analysed. The grain size of the samples was smaller than about 30  $\mu\text{m}$ . Four or five of the standards were included in each batch of 40 samples placed in the accelerator vacuum. The SG samples were analysed as unknown samples under routine conditions during one of the 5 periods. All samples were made of about 0.4 g of powder thoroughly mixed with one drop of polyvinylalcohol in a 2 % aqueous solution. A 1 mm thick pellet with a 15 mm diameter was made using a force of 20 kN in a hydraulic press.

## RESULTS AND DISCUSSION

### Precision

A total of 88 analyses were made of the six standards. When the total material was examined two apparent trends could be seen. During each of the 5 periods the reproducibility of the X-ray analysis was high, but the level from period to period had a total range of 13 %. These variations in the levels of the X-ray results could be explained by a disturbance from the battery-operated carbon filament, although leakage currents without beam were always less than 1 % of the beam current used. The disturbance appeared as a constant factor in the current integration giving different levels of quantification during each of the 5 periods. The average yield of the strontium peak of the AGV-1 standard, which was analysed 2 to 5 times during each of the 5 periods, was used for normalising the different levels.

TABLE 1. Average change in thick-target gamma-ray yield per 25 keV between 2.52 and 2.62 MeV. The change is relative to the yield at 2.55 MeV. Lines marked with \* have been used for quantification.

| =====   |              |            |
|---------|--------------|------------|
| Element | Energy (keV) | Change (%) |
| F       | 197 *        | 5.5        |
| F       | 110          | 5.5        |
| Na      | 440 *        | 4          |
| Na      | 1634         | 9          |
| Al      | 1014 *       | 16         |
| Al      | 843          | 37         |
| =====   |              |            |

For the gamma-ray analyses the spread within a period was larger and the levels of the periods had a pattern different from the those of the X-ray analyses. The sensitivity of the gamma-ray analysis to small beam energy variations was determined by analysing one of the standards at 5 different proton energies between 2.52 and 2.62 MeV. The average variations in the yield of different gamma-ray energies around 2.55 MeV are given in table 1. The different pattern of the gamma-ray yield variation could be explained by a 20 keV change in the proton energy after a recalibration. The major part of change in X-ray yield due to this proton energy jump is automatically included in the strontium peak normalisation. The standard deviations of both X-ray and gamma-ray peak areas after correction for the different charge integration levels and proton energy jumps are given in table 2. The average of the precision (one S.D.) for all X-ray peak areas with more than 5000 pulses, silicon and zirconium excepted, was 1.6 %. For the gamma-ray peak areas the average was 3.9 %.

TABLE 2: The precision obtained for different elements expressed as the relative standard deviation (%) of the number of pulses per  $\mu\text{C}$ . All elements with more than 5000 pulses in the peak are listed. The number of analyses for each standard is denoted by n.

|     | AGV-1 | GSP-1 | G-2  | GM  | DTS-1   | SiO <sub>2</sub> |                          |
|-----|-------|-------|------|-----|---------|------------------|--------------------------|
| n = | 19    | 12    | 12   | 10  | 17      | 18               |                          |
| F   | 6.1   | 3.3   | 5.2  | 5.4 | -       | -                | gamma<br>ray<br>analysis |
| Na  | 3.7   | 2.9   | 3.6  | 2.5 | -       | -                |                          |
| Mg  | -     | -     | -    | -   | 3.1     | -                |                          |
| Al  | 3.6   | 3.6   | 5.7  | 2.2 | -       | -                |                          |
| Si  | 6.1   | 4.8   | 4.2  | 4.8 | 8.1     | 4.9              | x-ray<br>analysis        |
| K   | 1.6   | 1.8   | 1.8  | 1.4 | -       | -                |                          |
| Ca  | 1.3   | 1.6   | 1.7  | 1.7 | -       | -                |                          |
| Ti  | 2.0   | -     | -    | -   | Cr: 2.0 | -                |                          |
| Fe  | 1.3   | 1.2   | 1.3  | 1.0 | 1.0     | -                |                          |
| Rb  | -     | 1.6   | 1.7  | 1.0 | Ni: 1.6 | W: 1.3           |                          |
| Sr  | 1.8   | 2.3   | 2.3  | -   | -       | -                |                          |
| Zr  | 2.2   | 7.6   | 11.6 | -   | -       | -                |                          |

### Calibration

To establish the true proton charge needed to obtain a certain strontium peak area in the AGV-1 standard, several analyses were performed with a thin carbon foil in front of the sample. This technique for the elimination of charge build-up did not affect the charge integration even at very low beam intensities. The elemental concentrations of the standards were then calculated using thick-target factors corresponding to a matrix composition equal to the reference values. The semi-empirical X-ray cross-section formula from Akselsson and Johansson (AJ)<sup>5)</sup> was used. X-ray absorption cross-sections and proton stopping powers were taken from Veigele<sup>6)</sup> and Northcliffe and Schilling<sup>7)</sup> respectively.

Ratios of obtained to nominal values of elements with low statistical uncertainty are plotted in fig 1. The experimental points from the thin-target calibration made in 1980<sup>2)</sup>, which

are based on the AJ cross-sections, have been included for comparison. The obtained thick-target ratios, calculated from these thin-target sensitivities, have small but systematic deviations from unity. Comparisons with the semi-empirical cross-section formula from Johansson and Johansson (JJ)<sup>8)</sup> and with the ECPSSR formula<sup>9)</sup> do not offer any explanation for the deviations. Calculations of thick-target factors using the JJ cross-sections gave differences of less than 1.5 % for elements with energies below 7 keV and a 3.3 % higher value at 25 keV. For proton energies between 1.5 and 2.5 MeV the X-ray energy dependence (and also the absolute levels) of the ECPSSR cross-sections agrees much better with the AJ values than the JJ values and the difference in thick-target factors using the ECPSSR cross-sections will probably be even smaller.

The large deviations in the aluminium and silicon ratios in fig.1 correspond to an increase of approximately 3 % in the thickness of the beryllium window of the chamber, but the significant deviation from the earlier thin-target calibration rather points to a systematic error in X-ray absorption cross-sections. Surface roughness effects may also be important, though the difference in yield from a pellet of ground quartz (containing 2000 ppm of tungsten) to a smooth surfaced quartz chip was small (<2 %). Although the silicon thick-target factors for the quartz are about 40 % lower and for the DTS-1 sample about 40 % higher than for the rest of the samples, which all have very similar matrices, the ratios of analytical to nominal values of silicon for these standards deviate less than a few percent. This implies a high usefulness of the data base for the absorption cross-sections used since systematic errors will partly be eliminated with the calibration procedure employed. Most data bases of proton stopping powers show very small differences in values and thus this parameter ought to give small uncertainties in the thick-target calculations. (For samples containing significant amounts of hydrogen the uncertainties due to different data bases will increase, though the uncertainty due to difficulties in establishing the hydrogen content might be more serious).

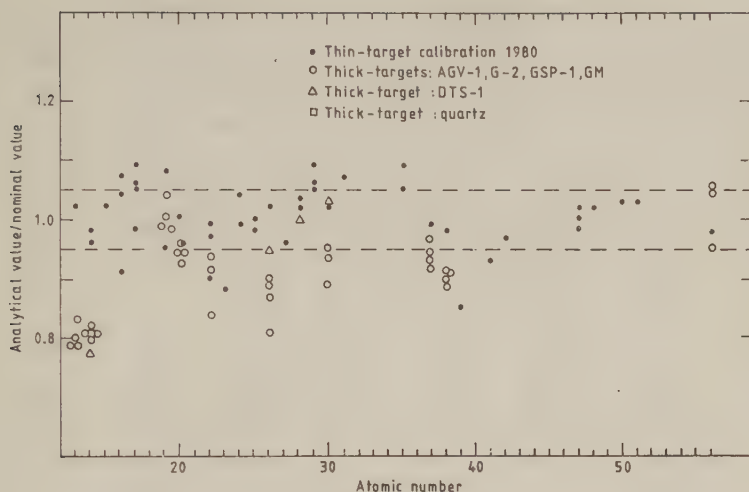


Figure 1: The ratios of analytical to nominal values for the elements Al, Si, K, Ca, Ti, Fe, Ni, Zn, Rb, Sr and Ba determined from PIXE-analysis of geological standards. Each point is the average of 10 to 19 analyses with standard deviations as given in table 2. Thick-target factors calculated from their reference compositions were applied. The dots are ratios from a previous calibration, where thin-target standards were analysed<sup>2)</sup>. All analytical values were determined with the semi-empirical cross-section formula for X-ray production given by Akselsson and Johansson<sup>5)</sup>.

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The ratios from the thick-target standards, seen in figure 1, were used for establishing apparent thin-target sensitivities, which will have corrections built into them for systematic errors in the matrix corrections performed with the data bases of references 6 and 7. The average values of these new sensitivities relative the normal thin-target sensitivities

based on the AJ cross sections are given in table 3. The validity of this calibration is, in principle, limited to silica-based rock material and the same type of calibration procedure will have to be done for other kinds of matrices if accuracies down to a few percent are to be reached.

TABLE 3: The ratios of thin-target sensitivities obtained from geological standards to the ones previously established<sup>2)</sup>. Relative standard deviations of the ratios are also given.

| Element | Ratio | S.D. | Element | Ratio | S.D. |
|---------|-------|------|---------|-------|------|
| Al      | 0.803 | 2.0% | Fe      | 0.89  | 5.2% |
| Si      | 0.803 | 1.7% | Zn      | 0.95  | 5.0% |
| K       | 1.005 | 2.3% | Rb      | 0.940 | 2.1% |
| Ca      | 0.942 | 1.4% | Sr      | 0.903 | 1.2% |
| Ti      | 0.90  | 4.6% | Ba      | 1.02  | 4.8% |

### Accuracy

The accuracy attainable can be estimated from the spread of points in figure 1. The standard deviations of the thick-target ratios in figure 1 are also given in table 3. The spread amounts to about 2 % (one S.D.) for most of the elements. This spread can be explained mainly by a sample composition differing from the reference values. E.g., the deviating points at Z=26,28 and 30 of the DTS-1 standard would only be explained by a 25 to 30 % too high value in the stopping power of magnesium, but e.g. the difference between the data used and the values given by Andersen and Ziegler<sup>10)</sup> is only 1 to 2 %.

To further evaluate the accuracy of this calibration the ten SG samples were analysed using the new calibration. The thick-target factors were calculated using an iterative procedure<sup>1)</sup>. The results of the analysis are presented in table 4. In the analysis of the SG samples the elements with a statistical variation below 3 % had an average difference from the reference values of 1.9 % (range 0.6 - 3.3). The homogeneity of the ten samples was very good. Only the zirconium values have a standard deviation significantly exceeding the statistical



TABLE 4: Mean values, MV, and standard deviations, SD, for all detected elements in ten duplicate SG samples. The uncertainty (one S.D.) due to pulse statistics, PS, is also given. Elements quantified by gamma-ray analysis are marked with +. The mean values, standard deviations, number of determinations, N, and methods used in establishing the reference values are also presented. Mean values are given in  $\mu\text{g/g}$  except when an element is expressed as the oxide.

| PIXE-PIGE results                |         |           |           | Reference values |           |    |              |
|----------------------------------|---------|-----------|-----------|------------------|-----------|----|--------------|
| Element                          | MV      | SD<br>(%) | PS<br>(%) | MV               | SD<br>(%) | N  | Method<br>&) |
| + Li                             | 18      | 16        | 30        | -                |           |    |              |
| + F                              | 1160    | 3.1       | 1.4       | -                |           |    |              |
| + Na <sub>2</sub> O              | 2.94 %  | 1.6       | 0.3       | 3.03 %           | 1.6       | 23 | AAS          |
| + MgO                            | 0.77 %  | 24        | 25        | 0.79 %           | 1.4       | 23 | AAS          |
| + Al <sub>2</sub> O <sub>3</sub> | 14.2 %  | 1.4       | 1.1       | 14.54 %          | 0.31      | 18 |              |
| SiO <sub>2</sub>                 | 67.2 %  | 1.1       | 0.6       | 68.66 %          | 0.26      | 18 | XRF          |
| P <sub>2</sub> O <sub>5</sub>    | <0.2 %  |           |           | 0.13 %           | 8.7       | 23 | SP           |
| K <sub>2</sub> O                 | 5.44 %  | 1.3       | 0.4       | 5.41 %           | 0.38      | 18 | XRF          |
|                                  |         |           |           | 5.29 %           | 1.2       | 6  | AAS          |
| CaO                              | 2.16 %  | 1.7       | 0.9       | 2.18 %           | 0.46      | 18 | XRF          |
| TiO <sub>2</sub>                 | 0.443 % | 3.2       | 1.7       | 0.48 %           | <2.1      | 18 | XRF          |
| Fe <sub>2</sub> O <sub>3</sub>   | 3.24 %  | 1.6       | 0.4       | 3.35 %           | 0.29      | 18 | XRF          |
| Mn                               | 423     | 4.1       | 6.0       | 413              | 6.5       | 20 | AAS          |
| Cu                               | 24      | 9.4       | 8         | -                |           |    |              |
| Zn                               | 61      | 5.8       | 3.3       | -                |           |    |              |
| Ga                               | 20      | 5.9       | 6         | -                |           |    |              |
| Rb                               | 180     | 1.5       | 1.2       | 180              | 2.2       | 4  | XRF          |
| Sr                               | 352     | 1.2       | 0.9       | 362              | 2.5       | 4  | XRF          |
| Y                                | 33      | 6.5       | 4.3       | 42               | 2.0       | 4  | XRF          |
| Zr                               | 342     | 9.5       | 1.1       | 368              | 2.2       | 4  | XRF          |
| Nb                               | 17      | 9.1       | 9         | 23               | 3.6       | 4  | XRF          |
| Ba                               | 1530    | 2.9       | 4.1       | 1470             | 0.7       | 4  | XRF          |
| Pb                               | 20      | 9.7       | 10        | -                |           |    |              |
| Th                               | 21      | 8.2       | 10        | -                |           |    |              |

&) AAS - Flame atomic absorption spectrometry  
 XRF - wavelength dispersive X-ray fluorescence  
 SP - spectrophotometry

variation. The variation in the silicon values is small (1.1 %), which means that the spread seen in table 2 (average 5.5 %) can be decreased by regular (e.g. each 40 samples) recalibration. The total mass analysed per sample is approximately 5 mg. Inhomogeneities at this level are the probable cause of the high standard deviation in the zirconium values both here and in table 2. In the latter case due to irradiation of different parts of the one pellet used per standard on different occasions.

### CONCLUSIONS

The combined PIXE-PIGE method has proved to be a reliable and accurate tool for determination of major and trace elements in thick samples. If uncertainties due to pulse statistics are neglected the precision in the analysis is better than 2 % even for non-conducting samples.

By using a calibration procedure where standards of composition similar to the unknown samples are used, it is possible to establish apparent thin-target sensitivities, which will have corrections for systematic errors in the matrix corrections included. These apparent thin-target sensitivities will be valid only for the type of matrices used in the calibration. In this way an accuracy of 2 or 3 % in elemental determinations may be obtained.

Powdered geological material with grain sizes smaller than about 30  $\mu\text{m}$  was compressed into pellet samples, which, analysed at the 5 mg level, showed a homogeneity better than about 2 % for most elements. Deviations from reference values at levels around 10 % did occur for some of the samples used here and larger deviations have been seen in later experiments also for other international standards. Since these deviations are most certainly due to inhomogeneities it is advisable to use at least 4 or 5 pellets of each standard sample in performing a calibration of the type described here.

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## Part VII. Analysis of thick samples

### PIXE ANALYSIS OF SAMPLES OF INTERMEDIATE THICKNESS

Lars-Eric CARLSSON, Klas G. MALMQVIST, Gerd I. JOHANSSON and K. Roland AKSELSSON

*Department of Nuclear Physics, Lund Institute of Technology, Sölvegatan 14, S-223 62 Lund, Sweden*

A procedure for making accurate matrix corrections in PIXE analyses of samples of intermediate thickness has been developed. The transmission of a collimated X-ray beam through different parts of the sample is measured with a Si(Li) detector to determine the thickness and shape of the sample. Experiments have been performed using uniform polymer foils doped with known concentrations of different elements and with thicknesses ranging from 1.5 to 11 mg/cm<sup>2</sup>. The results from these samples indicate that the accuracy of the correction procedure is better than 5%. The correction procedure has been applied to, e.g., samples obtained in single orifice cascade impactors.

#### 1. Introduction

Particle Induced X-ray Emission analysis is preferentially applied to thin samples for which particle slowing down and X-ray attenuation can be neglected. In aerosol analysis, a major field of application for the PIXE method, the combination of small low-volume air samplers and PIXE constitutes a powerful technique for the determination of elemental composition. In particular, the information on aerosol size distributions attainable with the single orifice cascade impactor has been found to be essential in atmospheric aerosol studies [1,2] as well as in characterisation of work environment aerosols [3,4]. Since aerosol concentrations may vary by several orders of magnitude during and between sampling periods, the mass of the sample collected in the cascade impactor is very difficult to predict. The samples may reach thicknesses in the range of 2 to 5 mg/cm<sup>2</sup> [5]. These samples cannot be regarded as being either infinitely thick or very thin when analysed with PIXE. To allow for corrections for particle slowing-down and X-ray attenuation the accurate thickness has to be determined. Also possible non-uniformity of the sample has to be considered.

There are many other applications in biology and medicine in which samples of intermediate thicknesses occur, e.g., thin sections of dried tissue may not be regarded as being infinitesimally thin when analysed with PIXE.

##### 1.1. Matrix corrections

When calculating the elemental concentrations in a sample of non-negligible thickness, the decrease in

ionisation cross section due to the slowing-down of particles in the sample and the increased absorption of the X-rays generated inside the sample have to be taken into consideration. In the present work, X-ray attenuation cross sections and stopping powers of particles have been taken from recent compilations of data [6,7]. In calculating the correction factors, the polynomial fit to ionisation cross sections given in ref. [8] has been used.

To make accurate corrections, the major constituents of the sample have to be determined; this will be discussed in detail later.

#### 2. Experimental

Thicknesses of samples are determined by measuring the transmission of Ag L<sub>α</sub> or K K<sub>α</sub> X-rays through the sample. The X-rays are produced by irradiating a thick, solid Ag target or a thick K<sub>2</sub>CO<sub>3</sub> pellet with 2.55 MeV protons from the 3 MV tandem accelerator in Lund [9]. The arrangement used is shown in fig. 1. Within a frame fixed on the housing of the Si(Li) detector are mounted an interchangeable X-ray collimator and a moveable sample holder. The smallest tantalum collimator has a diameter of 0.15 mm. The transmission through the sample could thus be measured with a spatial resolution of better than 0.20 mm. Scanning a sample in two perpendicular directions is possible using micrometer screws.

The X-rays transmitted are detected in the Si(Li) detector and the area of the characteristic X-ray peak is measured and normalised to the integrated beam charge for each scanning step. The shape and thickness of the sample can thus be determined. For small

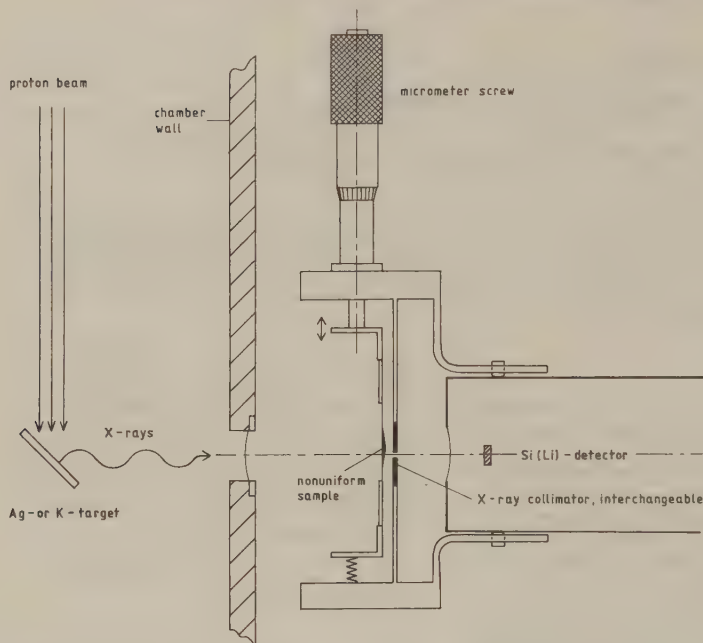


Fig. 1. Experimental arrangement used for determining the thicknesses and shapes of non-uniform samples (not to scale). The small tantalum collimator can be exchanged for larger ones when measuring the thicknesses of large homogeneous samples.

samples on a thin backing, separate transmission measurements are made for the backing and the sample. By repeated measurements of the backing material, possible inhomogeneities may be estimated.

The intensity of the characteristic X-rays from this source arrangement is quite high allowing measurement times for typical samples in the range 30–100 s for each scanning step. For measurements of thicknesses of flat uniform samples a larger X-ray collimator could be used to increase the speed of analysis.

### 2.1. Cascade impactor samples

In our laboratory we use the Battelle-type single orifice cascade impactor with an air-flow of 1 l/min [10]. For atmospheric aerosols heavy loadings may occur on the last three stages of the impactor corre-

sponding to 50% cut-off aerodynamic diameters of 1, 0.5 and 0.25  $\mu\text{m}$  respectively. Overloading of the last two stages may easily occur during sampling in many work environments, where the aerosol concentrations are much higher than in the atmosphere and particle diameters smaller than 1  $\mu\text{m}$  are predominant. To avoid this, it is necessary to restrict the sampling time which limits the amount of information from the less loaded stages.

The nozzles of the last two stages produce samples with diameters in the 0.5 to 1.5 mm range. By inspection under a light microscope and by scanning transmission measurements, impactor samples have been found to be approximately cone-shaped. The thickness at the centre of the cone is determined in the position at which the lowest count rate of the transmitted X-rays occurs and a computer code is used to



calculate the necessary corrections due to proton slowing-down and X-ray attenuation.

The matrix composition of a sample is determined in the following way: The medium- to high- $Z$  constituents ( $Z \geq 14$ ) are measured in the PIXE analysis. The transmission measurements give the total sample mass and then the fraction of low- $Z$  elements may be estimated. The use of complementary analytical techniques, e.g. elastic scattering of particles or particle-induced gamma-rays [3], would extend the number of matrix elements which can be determined thus permitting more accurate corrections.

Using the initial composition determined, the PIXE results are corrected for proton slowing-down and X-ray attenuation. These more accurate results are then used to recalculate the composition of the matrix, thus yielding improved correction factors. This iteration may then be repeated several times to improve the PIXE results.

For routine corrections of impactor samples a simplified procedure is often adequate. From only a few samples the fraction of low- $Z$  elements is calculated and is then assumed to be the same for all the samples in a batch. Corrections can then be made without performing the transmission measurements since the total sample mass may be calculated from the PIXE analysis using the iterative technique described above.

## 2.2. Determination of correction accuracy

To evaluate the accuracy of the correction procedure, homogeneous samples with known compositions and thicknesses were used. Polymer foils, homogeneously doped with organometallic compounds to concentrations of approximately 1% [11],

were kindly supplied by T.G. Dzubay. Three foils, each 37 mm in diameter, doped with Pb, V and Ni respectively, were cut into 12 pieces. From these 12 pieces, 6 samples were made: 3 samples with single layers and one each with 2, 3 and 4 layers put together. In this way sample thicknesses ranging from 1.5 to 11 mg/cm<sup>2</sup> were obtained.

The metal contents of the samples were measured using PIXE analysis. The elemental uniformity of the samples as determined from the analyses of the three single layer foils was better than 2%. The PIXE analysis was performed using the experimental arrangement described in ref. [9].

## 3. Results and discussion

### 3.1. Correction procedure

The thicknesses of the samples, as determined from the transmission measurements of K  $K_{\alpha}$  X-rays, were in good agreement with the gravimetrically-determined values given by Dzubay et al. (see table 1). The experimental values of the metal content in the foils before and after corrections are plotted against the experimentally-determined foil thicknesses in fig. 2. The points corresponding to the one-layer samples are the averages of the three samples used. The straight lines are fitted to the corrected values, constraining the lines to pass through the origin.

Any systematic error in the proton stopping power data used would result in a deviation from the straight lines in fig. 2. This would be most noticeable for the Pb  $L_{\alpha}$  and Ni  $K_{\alpha}$  lines. Similarly, a systematic error in the X-ray attenuation coefficients would appear as a deviation from a straight line for the Pb

Table 1  
Results from thickness determination using the arrangement in fig. 1. The polymer foils from ref. 11 have been measured and the results are given in mg/cm<sup>2</sup> and compared with the results from ref. 11, which have been obtained using gravimetric analysis.

| Matrix<br>Dopant | Polystyrene<br>Lead |                     | CAP<br>Vanadium |                     | CAP<br>Nickel |                    |
|------------------|---------------------|---------------------|-----------------|---------------------|---------------|--------------------|
|                  | This work           | Ref. 11             | This work       | Ref. 11             | This work     | Ref. 11            |
| Number of foils  |                     |                     |                 |                     |               |                    |
| 1                | 2.75                | 2.72                | 2.59            | 2.56                | 1.69          | 1.65               |
| 2                | 5.43                | 5.44 <sup>a)</sup>  | 5.08            | 5.12 <sup>a)</sup>  | 3.29          | 3.30 <sup>a)</sup> |
| 3                | 7.93                | 8.16 <sup>a)</sup>  | —               | —                   | 5.02          | 4.95 <sup>a)</sup> |
| 4                | 10.57               | 10.88 <sup>a)</sup> | 9.46            | 10.24 <sup>a)</sup> | 6.41          | 6.60 <sup>a)</sup> |

<sup>a)</sup> The thicknesses of multilayer samples have been calculated by simply multiplying the thickness of a single foil (from ref. 11) by the number of foils.

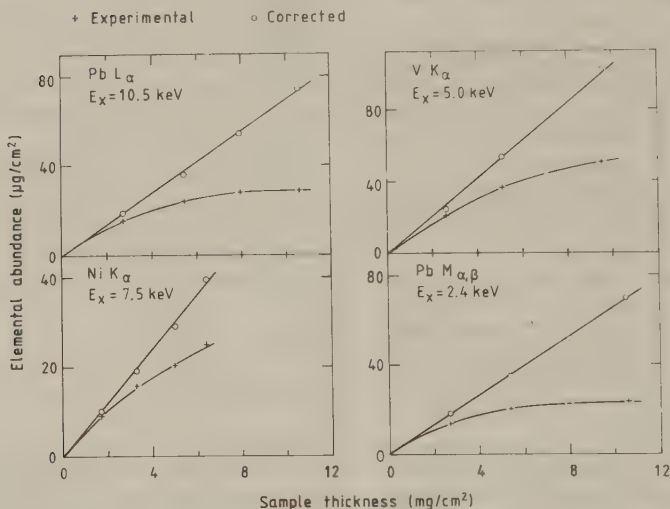


Fig. 2. Elemental abundances obtained before (+) and after (o) correction for proton slowing-down and X-ray attenuation, plotted against the sample thicknesses as determined with the arrangement in fig. 1. The matrix for the V- and Ni-containing foils is cellulose acetate propionate (Eastman CAP-20) and for the Pb-containing foil polystyrene.

M-case. No experimental value in the diagrams in fig. 2 deviates from the straight lines fitted by more than 5%. Hence, the accuracies of the correction factors calculated are better than 5%, since part of the deviation is due to the statistical errors.

From the above it follows that possible systematic errors in the tabulated values of X-ray attenuation cross sections and proton stopping powers are small. This conclusion is valid for the matrix elements in the

polymer foils used, i.e., hydrogen, carbon and oxygen. These elements are, however, major constituents of many different types of samples.

In the transmission measurements, the reproducibility in the determination of the number of transmitted characteristic X-rays using 30–60 s counting times was 1.2% (one standard deviation). From this value the precision in the thickness determination, expressed as one standard deviation, has been calculated and is shown in fig. 3 as a function of the sample thickness. The corresponding precision in the correction factor is also plotted.

### 3.2. Impactor development

When using this experimental arrangement (fig. 1) to study the thickness and structure of cascade impactor samples, it became obvious that corrections were indeed necessary and that these were sometimes quite large. For some of these unintentionally overloaded samples, too low values of elemental mass will result although accurate corrections are made using the procedure described above. The reason for this is actual physical loss of particles from the impactation surfaces during sampling.

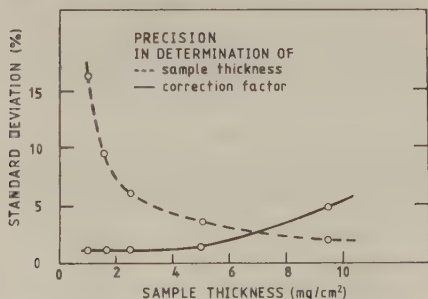


Fig. 3. The precision in sample thickness determination and correction factor calculation for the polymer foils used, as a function of sample thickness (one standard deviation).

The collection characteristics of the impaction plate are severely altered when too much material is collected within too small an area. These findings initiated a development of the Battelle impactor to increase the area of impaction on the last stages. Details of this development are given in ref. [12].

#### 4. Conclusions

The developed arrangement (fig. 1) constitutes a very useful experimental facility for the detailed study of the structure of non-uniform samples. Using it to determine shape, thickness and sample composition, very accurate corrections (better than 5%) can be made in PIXE analysis of samples of intermediate thickness.

We are very grateful to Mr. H. Kaufmann, Florida State University, Tallahassee, USA for his active participation in the initial phase of this work and to

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## PIXE - A NEW TOOL IN QUANTITATIVE DERMATOLOGY

K.G. Malmqvist<sup>\*1</sup>, L.-E. Carlsson<sup>1</sup>, K.R. Akseleson<sup>2</sup> and B. Forslind<sup>3</sup><sup>1</sup>Department of Nuclear Physics, Lund Institute of Technology, Sweden<sup>2</sup>Department of Work Environment Technology, Lund Institute of Technology, Sweden<sup>3</sup>Department of Medical Biophysics, Karolinska Institute, SwedenAbstract

Proton-Induced X-ray Emission analysis (PIXE) constitutes a method for trace element analysis characterized by multielemental capability, detection limits in the low ppm-range and size resolution down towards a micrometre. In applications where the sensitivity of the Electron-Induced X-ray Emission (EIXE) analysis is not sufficient and where a spatial resolution not better than a few micrometres is required, the PIXE technique provides a powerful tool. In this paper properties of the PIXE method are demonstrated by quantitative results from three different samples of dermatological interest. Firstly, mercury results from a longitudinal scan of a single hair strand from a mercury poisoned person are shown. With a spatial resolution of one or a few millimetres very fast scans may be performed on hair strands giving information on time and magnitude of intoxication or other exposures, as well as deficiencies. Secondly, results are given from a radial scan with a beam width of 4  $\mu\text{m}$  on hair from a person exposed to high amounts of iron. The calcium, iron and zinc distributions but not the sulphur and potassium distributions show narrow peaks of concentration (less than 4  $\mu\text{m}$ ) about 15  $\mu\text{m}$  from the surface of the hair. Further investigations have to be performed in order to interpret these data. Thirdly, the depth profiles in skin of some elements were measured with a beam width of 10  $\mu\text{m}$ . The results show significant increases in sulphur, calcium and zinc concentrations and significant decreases in phosphorous and potassium concentrations at the skin surface, i.e. in the stratum corneum.

**KEY WORDS:** Proton microprobe, proton-induced X-ray emission, PIXE, external beam, energy-dispersive, electron microprobe, dermatology, hair, skin, cryosectioning

Department of Nuclear Physics, Lund Institute of Technology, Sölvegatan 14, S-223 62 LUND, Sweden

(046) 107684

Introduction

Although it has been possible to collect data from skin and its appendages through biochemical and biophysical methods as well as through light and electron microscopy, information on the elemental content in situ has only recently been available (27). Energy-dispersive X-ray microanalysis with an electron microprobe (EMP) provides such data collection on frozen hydrated or freeze-dried sections obtained after freeze-quenching of the specimens. However, the EMP is not a trace element method and important electrolytes such as calcium and magnesium are barely detectable above the significant background obtained by analysis. In the search for methods allowing the detection of elements at concentrations close to trace element levels, i.e. 10-500 ppm, we have investigated the analytical potential of PIXE (proton induced X-ray emission analysis). The high sensitivity and rapidness of PIXE makes it an attractive tool in many applications of trace element analysis. For detailed descriptions and reviews see Johansson and Johansson (14) and Khan and Crumpton (16).

Longitudinal hair scan

The technique of longitudinal scanning of hair in a helium atmosphere with the PIXE technique provides an accurate, non-destructive and rapid method for detecting enhanced concentrations of toxic elements, which may be due to acute poisoning, with applications in, for example, forensic science (11). Furthermore, it may be of great use in analysing hair samples for extensive epidemiological studies when occupational or environmental pollution is suspected.

Inherent in the PIXE technique is the capability of estimating surface to bulk distributions of elements in thick samples (1). In the case of hair a repeated analysis at 2 or 3 different proton energies have been used to roughly calculate the surface to bulk ratios of several elements (21). This technique may be of use for separating any possible exogenous contamination from endogenous components.

Several investigations of the spatial distribution of elements in hair have been performed using PIXE analysis (15,25). Another suitable technique is the use of X-ray fluorescence analysis. Toribara et al (23) used molybdenum-

filtered radiation from a molybdenum X-ray tube and a spatial resolution of a few mm in studies of trace elements in hair.

A similar technique to obtain time records of trace element concentrations would be to use another appendage of skin, i.e. nails. Samples can easily be collected from, e.g. occupationally exposed workers and the elemental distributions along the nail may be determined by direct PIXE analysis. This technique has been used with neutron activation analysis in a case of repeated arsenic poisoning (10). Preliminary attempts have been made at our laboratory to sample thin strips from nails and to scan the strips longitudinally. Sampling on workers exposed to arsenic is at present being planned.

#### Microprobe analysis

In common with the electron beam in an electron microscope the proton beam may be finely focused by magnetic or electrostatic means, allowing elemental analysis with high spatial resolution. A detailed review of the properties and possibilities of proton microprobes has recently been given by Martin (22).

There is a strong trend towards an increasing number of laboratories developing microbeams for applications in different fields. In a recent conference in Namur, Belgium, many laboratories presented their progress (8). Cookson and Pilling (6) and Bos et al. (3) have reported interesting results from cross sectional scans of hairs performed on thick slices of hair strands. Bischof et al. (2) have reported depth profiles of some elements in skin showing strong variations in concentration introduced by the presence of a hair root. In the present communication we report the first quantitative results from a freeze-quenched, freeze-sectioned and freeze-dried thin section of hair obtained with a finely focused microbeam of protons as well as quantitative elemental profiles on normal human skin provided under the same conditions. Furthermore, PIXE data on single hair strands obtained with a macrobeam will be discussed in this context.

#### Materials

##### Hair

One hair sample was obtained from a woman aged 36, who had been exposed to very high amounts of iron in domestic water supply (13 mg/l). Hair fibres were plucked ensuring that anagen fibres were obtained and sent by post without any further preparation. Short pieces of hair (2-4 mm) were cut from the first 1.5 cm (root end) of the fibre and placed in carboxymethyl cellulose, swelled in doubly distilled water and immediately quenched-frozen in liquid nitrogen. Thin sections (< 5 µm) were obtained with a specimen knife of glass at a temperature of -100 degrees C by manual sectioning on a Reicher OMU3 F2 cryoultramicrotome. The sections were collected on a 2 µm thick sheet (Kimfol<sup>R</sup>) and fixed to it by thawing and subsequent air drying. The plastic sheet carrying the sections were then transferred to circular specimen holders allowing free passage of the protons after interaction with the specimen.

A second hair sample for analysis with a macrobeam was collected from a victim of mercury

poisoning. The hair was stretched and glued, self-supporting, without any pre-treatment onto a plastic frame (20 x 200 mm<sup>2</sup>). The length of the hair to be analysed was 150 mm.

##### Skin

Skin samples were taken by punch biopsy from the lower arm of a healthy volunteer without any previous local anesthesia. Semi-thick sections (10 - 30 µm) were cut at -25 degrees C on a Leitz cryostat and the sections were subsequently freeze-dried in the cryostat at -25 degrees C for 6 hours. Each freeze-dried section was then mounted on a 2 µm thick sheet (Kimfol<sup>R</sup>) in the circular specimen holder designed for our instrument. To prevent the uptake of water before analysis the specimens were stored over Drierite<sup>R</sup>.

#### Methods

For the PIXE analysis protons with an energy of 2.5 MeV were delivered by an electrostatic accelerator (Pelletron 3UDH). The characteristic X-rays produced on impact by the protons on a sample are detected by an 80 mm<sup>2</sup> Si(Li) detector (Kevex; nominal resolution 158 eV at 5.9 keV) placed at an angle of 135 degrees relative to the beam. The presence of all elements heavier than sodium can be determined simultaneously by storing the X-ray events in a multichannel analyzer (Nuclear Data 6620) and transferring the X-ray spectra to magnetic tape for subsequent evaluation. For routine analysis a beam diameter of 1 to 10 mm is used. Precision and accuracy are normally better than 5 to 10 % (13,5).

##### External beam PIXE

For macrobeam analysis of hair a tantalum beam collimator of 2x4 mm<sup>2</sup> was used with the long side perpendicular to the hair. The proton current was kept below 40 nA. To reduce heating and charge build-up during irradiation the hair was placed in an atmosphere of helium (pressure 50 kPa). The proton beam was extracted from vacuum through a thin polyimide foil (Kapton<sup>R</sup>, 7.5 µm). After passing through the hair the proton beam leaves the target chamber through another foil to a high-vacuum Faraday cup where the current is measured. Detection of X-rays is performed as described above.

The plastic frame with the hair was placed in a tray drawn by a linear stepping motor (Aiprax L92121-P2) allowing a spatial resolution of 50 µm. After complete analysis of one section of the hair the next section is moved into the beam by remote control (computer controlled).

The hair strands represent samples of non-negligible thickness (diam: 40 - 110 µm) and to be able to calculate accurate elemental concentrations a surface barrier detector is used to measure the energy distribution of scattered protons. From this particle energy spectrum it is possible by a special algorithm, to determine the hair diameter (19,20).

##### The proton microprobe (PMP)

An adjustable collimator is situated after the accelerator at a distance of 4 metres from the sample chamber. With the aid of micrometre screws the opening of this object collimator can be set separately from 10 to 1500 µm in two perpendicular directions. With 4 quadrupole magnets positioned



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about 0.3 m in front of the sample, beam dimensions down to a few micrometres may be attained by focusing. In these experiments only two magnets were used. The experimental demagnification factors with this doublet configuration were 8 and 38 in the horizontal and vertical planes respectively.

The sample holder can be moved by remote control in steps of 1  $\mu\text{m}$  or more in both horizontal and vertical directions. To identify and position the samples they are illuminated by an external light source of high intensity through a system of mirrors and windows. Viewing is carried out using a stereo microscope (Nissho ZN 240 with a zoom lens and a maximum magnification of 180x).

The chamber vacuum was about  $10^{-5}$  torr and the highest obtained proton current was approximately 3  $\text{pA}/\mu\text{m}^2$ . With a pair of extra quadrupole magnets situated in front of the object collimators the proton density can be strongly increased although the divergence of the beam and thus the aberration in the lenses will increase and only for larger beam sizes this will be an advantage.

For the skin sample the beam was focused in a spot of  $10 \times 100 \mu\text{m}^2$  oriented with the long side parallel to the skin surface and the irradiation was started about 35  $\mu\text{m}$  into dermis. The sample could be moved perpendicular to the beam (in steps of 8  $\mu\text{m}$ ) to analyse the various epidermal strata. Each spot was irradiated for 300 to 500 s.

For the elliptical hair section (see upper left part of fig.3) the beam was focused on an area of  $4 \times 15 \mu\text{m}^2$ . The beam profile parallel to the short side, measured by scanning the edge of a thin, self-supporting tantalum foil, is shown in fig.1.

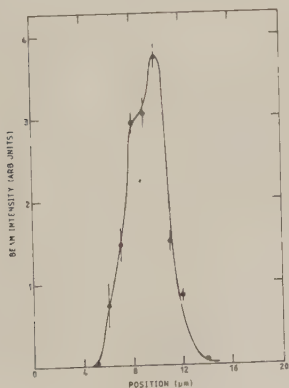


Fig.1. Vertical beam profile as determined by scanning the edge of a thin self-supporting tantalum foil. The error bars represent one S.D.

The hair sample was moved with 3  $\mu\text{m}$  increments and the irradiation times were approximately 500 s per point. The zone irradiated by the proton beam formed a 15  $\mu\text{m}$  wide strip passing through the centre of the hair. Unfortunately the strip at one side of the hair passed a part of the cuticula which was partly damaged during sectioning.

For both the hair and the skin sample no corrections due to proton slowing down and X-ray absorption were required for elements heavier than silicon.

### Results and discussions

#### Longitudinal hair scan

In fig.2 the concentration of mercury along the hair strand is plotted. The concentration is calculated by assuming a constant sulphur concentration of 4.3 %. This value was obtained from PIXE-analysis of a total of 40 hair strands, which were collected from Caucasian as well as Asian subjects (20).

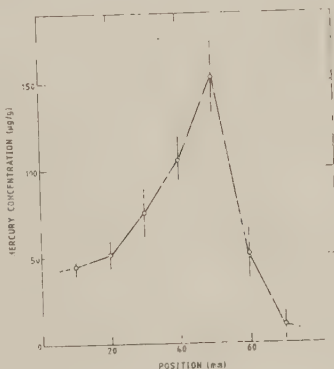


Fig.2. Mercury concentration along a single human hair strand as determined by macrobeam PIXE analysis. Origin corresponds to the root end. The donor of the hair was a victim of mercury poisoning. Irradiations were carried out in a helium atmosphere with a beam of 2.5 MeV protons with a beam collimator of  $2 \times 4 \text{ mm}^2$  and a current density of less than 5  $\text{nA}/\text{mm}^2$ . The solid lines are drawn to guide the eye and the error bars show statistical uncertainties of one S.D.

Since only 2 mm sections were analysed every 10 mm along the hair the spatial resolution may easily be improved without any reduction of the sensitivity - thus facilitating determination of elemental variations with a time resolution of less than one week. The detection limit for mercury in this set-up is around 8  $\mu\text{g}/\text{g}$  using 100 s irradiation time.

### Cross sectional hair scan

The results from the transverse scan of the thin hair section are shown in fig 3. In calculating the elemental concentrations the sulphur concentration has been assumed to be 4.3 % as determined by Li and Akselsson (20). Part of the cuticula was damaged during sectioning (see fig.3) and results from this part have consequently been excluded in the diagram. For orientation, the centre of the elliptic hair cross section as determined by a light microscope is indicated.

In fig.3 the sulphur distribution is given in arbitrary units as an index of mass distribution, showing a rather smooth distribution over the whole hair cross section. The small variations in the sulphur distribution cannot be explained by pulse statistics but rather by the structure of the hair, i.e. a looser arrangement of the cells in the centre.

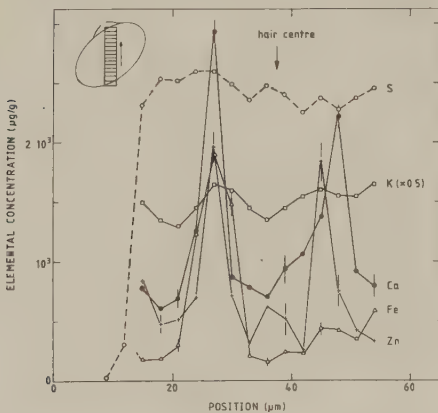


Fig.3. Elemental distributions across the thin section of a human hair. The scanning was performed according to the drawing in the upper left corner. Due to the damage caused during sectioning no values are given for the upper edge of the hair section. The analysis was performed with a proton microbeam of  $4 \times 15 \mu\text{m}^2$  and a current of about 200 pA. The broken line shows the sulphur distribution in arbitrary absolute units and does not refer to the ordinate scale. The solid lines are drawn to guide the eye and the error bars show the uncertainty due to pulse statistics (one S.D.).

For potassium the distribution is rather constant and the concentration rather high compared with the values in the literature, normally citing potassium concentrations well below 1000 ppm (17). For the elements calcium, iron and zinc the distributions are quite different with sharp and high maxima occurring approximately symmetrically around the hair

centre, about  $15 \mu\text{m}$  inside the edge of the hair section. In the remaining parts the levels are rather constant with variations approximately within the statistical uncertainties. However, for iron no maximum is found in the right part of the diagram. Since the spatial resolution of the proton beam is limited (FWHM =  $4 \mu\text{m}$ ) the maxima may appear wider than their actual widths. Thus if the widths of the maxima are considerably less than  $3 \mu\text{m}$  the peak concentrations are considerably higher than indicated in fig.3.

In a few other investigations, a PMP has been used for cross sectional scans of hairs (6,12). In their works they used a beam twice as broad as the one in the present work and the hair sections analysed were infinitely thick for the protons. In their investigations maxima were found for several elements but normally they occur at the hair surface (the edge of the section) and are probably caused by exogenic contamination. However, in the report by Houtman et al. (12) calcium and zinc distributions for a few hairs were found to peak somewhat inside the hair similarly to that found in the present work. They explain this by exogenic contamination and diffusion followed by a superficial extraction. The smoothened peaks due to their limited spatial resolution make this a plausible explanation. In our case the very sharp maxima can hardly be explained in this way. The possibility of a contamination transferred to our sample during processing cannot be totally excluded but the probability is very small that the contamination would occur in such a symmetrical fashion as found in fig.3. Since the donor of the hair is suspected to suffer from a too heavy exposure to iron from domestic water supply the elevated levels of iron and several other elements might be explained by a concomitant endogen uptake as well as contaminant cosmetic care.

Apart from the elements given in fig.3 chlorine and copper were also found to be present. In fig.4 is shown a PIXE spectrum formed by summing the spectra obtained in the transversal hair scan.

The detection limits attainable in PIXE analysis depend on the beam intensity and the solid angle of the X-ray detector. However, an upper limit for the beam intensity is set by the heating induced in the sample. By using a PMP in beam scanning mode analysis (7,18) the average irradiation intensity will be much lower and the beam current can thus be considerably increased without any excessive heating of the sample. Also the sample thickness is of importance in regard to the detection limits. A thick sample ( $> 12 \text{ mg/cm}^2$  for 2.5 MeV protons) gives a higher yield, but also absorbs significantly more of the beam energy. To perform accurate matrix corrections the composition must be known. In thin samples no, or at least very small, corrections for X-ray self-absorption are required, but when calculating the elemental concentrations in a thin sample the sample thickness ( $\text{mg/cm}^2$ ) must be known.

The proton microprobes, when used with small beam areas, can usually deliver only a modest beam intensity and if this is a limiting factor the thick sample with its higher yields will give the lowest detection limits. In the case of samples of dermatological material the optimal thickness is

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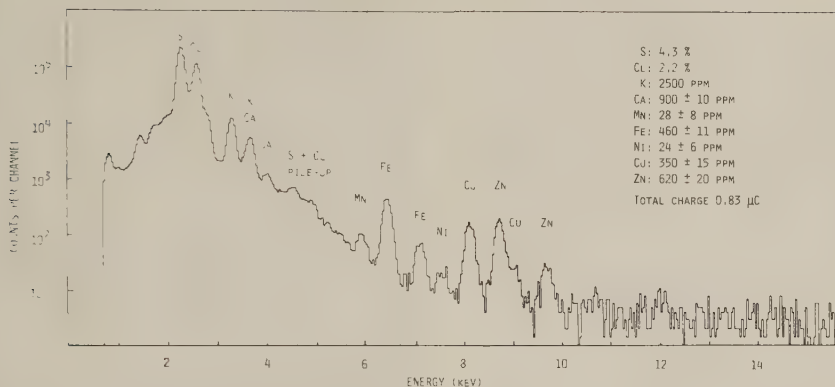


Fig.4. PIXE spectrum formed by summing the individual spectra obtained when scanning a thin human hair section. The total accumulated charge of protons was 0.83 μC. The elemental concentrations and uncertainties (one S.D.) are determined from this spectrum.

estimated to be about 3 mg/cm<sup>2</sup>. With a sample thickness of 3 mg/cm<sup>2</sup> only 18 % of the beam energy is absorbed by the sample, but the yield will be more than 55 % of that of a thick sample. With this sample thickness a surface barrier detector can be used to measure the energy of scattered protons, from which the sample thickness can be calculated. Since the yield of the <sup>27</sup>Al(p,p<sup>γ</sup>)<sup>27</sup>Al reaction is very sensitive to the proton energy, it is also possible to use the intensity of induced gamma rays from a beam stop of aluminium to determine the proton energy loss in the sample and thus monitor the sample thickness (4).

If a sample contains very high concentrations of elements with atomic numbers below 28 an X-ray filter with a hole can be used to decrease the solid angle of low-energy X-rays for the detector rather than decreasing the beam current. In this way a high sensitivity is maintained for heavier elements, while keeping the detector count rate at a suitable level.

#### Microprobe scan of human skin

In figs. 5a and b are shown the elemental depth distributions in the human skin sample.

The sample thickness (mg/cm<sup>2</sup>), which is needed when calculating the elemental concentrations has been determined by comparison of the intensity of the bremsstrahlung continuum for different positions with that of the supporting foil, which has a known thickness (0.18 mg/cm<sup>2</sup>). This continuum is proportional to the thickness of the irradiated material (24). The broken line in the figure shows the determined mass distribution with its maximum value in the dense stratum corneum. The zones of epidermis and dermis as determined visually in a light microscope are

indicated in the figures. As can be seen in the diagrams the statistical uncertainties are very small for the elements phosphorous, sulphur, chlorine and potassium which occur in high concentrations in skin. For calcium the uncertainties are more important due to overlapping between the large potassium K-beta peak and the calcium K-alpha peak. The trace elements iron and zinc are plotted in fig.5b.

The phosphorous concentration, which is probably correlated to the phospholipids of the living cells, is low in dermis and has a maximum in the lowest part of the epidermis, stratum germinativum, where the cells are formed. The phosphorous concentration further out decreases slowly to show very low values in the stratum corneum where the phospholipids in the living cells are replaced by ceramides in order to form the barrier of the horny layer (28). The sulphur distribution shows only smooth variation with a slight maximum in the border region between dermis and epidermis but with a significant increase in the stratum corneum.

The potassium concentration is rather constant in the scanned section except in the outer part where it decreases in a similar fashion to phosphorous showing very low values in the stratum corneum. Chlorine is high in the dermis and decreases to a level which is constant through the whole of the epidermis.

Calcium is below the detection limit in the inner half of the epidermis, but the calcium distribution shows a steep increase in the outer part with a maximum at the surface of the stratum corneum. This may be due to an endogenic increase in the horny layer but the shape of the distribution, reminiscent of a diffusion gradient, may also be explained by external contamination from, e.g. domestic water supply with a high calcium content.

In fig.5b it is clear that lower detection limits would be required to reach any decisive conclusions regarding the distribution of the trace elements. However, both zinc and possibly iron seem to increase in a fashion similar to

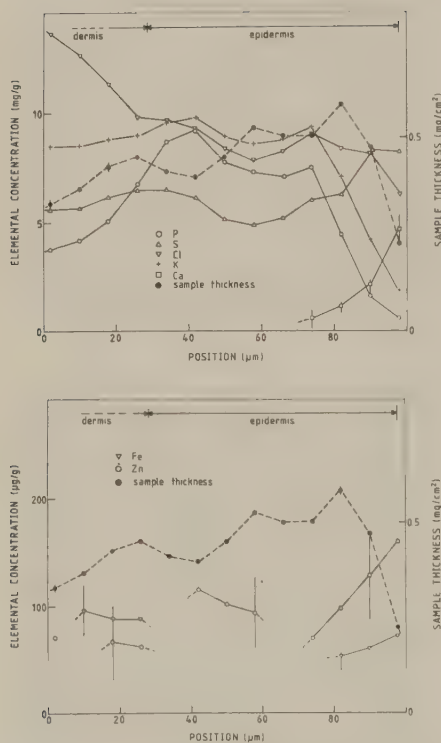


Fig.5 a/ Elemental distributions in normal human skin as determined by a proton microprobe with a beam area of  $10 \times 100 \mu\text{m}^2$  having the long side parallel to the skin surface. The broken line shows the sample thickness (right scale) as determined from the background in the PIXE spectra. The solid lines are drawn to guide the eye and the error bars represent the uncertainties due to pulse statistics (one S.D.).  
 b/ As fig.5a except that the trace elements Fe and Zn are sometimes below the detection limits and are then not marked. Detection limits for the two elements are between 30 and 50 ppm. Error bars showing one S.D. are occasionally given to show the magnitude of the uncertainty.

calcium in the stratum corneum, indicating possible exogenic contamination and diffusion. By improving the detection limits by a factor of five (to about 5 to 10 ppm), which may be achieved by increasing the beam intensity, irradiation time and the solid angle of the X-ray detector (see also the section "Crosssectional hair scan"), the distribution of several metals, such as chromium, manganese, iron, nickel and copper, may be determined. This is of interest in, e.g. occupational dermatology, in which mechanisms of uptake of metals through skin is very important.

The results from this study show agreement with those of e.g. Forslind et al (9) who studied the elemental distribution of several elements in guinea pig epidermis by the EMP. The distributions of the elements phosphorous, sulphur and potassium behave similarly to ours, while some elements, sodium and magnesium, were not detected in our study. In their investigation Bischof et al. (2) studied the whole skin (0.5 - 1 mm thick) with a rather limited spatial resolution. Interesting results, however, were obtained in this scan when passing a hair follicle deep in dermis. It was shown that sulphur, chlorine and zinc concentrations were high in the hair cortex, while the phosphorous concentration was high in the follicle region surrounding the hair strand, where cell formation takes place.

As far as detection of the lightest elements are concerned there exist no other restrictions in PIXE-analysis than in the other X-ray techniques. Except for a thin detector window it is also required to keep the sample very thin to reduce self-absorption of the very soft X-rays from elements like sodium and magnesium. However, for high concentrations of sodium and rather thick samples it is possible to use a complementary technique simultaneously with PIXE-analysis. By using 2.5 MeV protons and detecting gamma rays from the inelastic reaction  $^{23}\text{Na}(p,p'\gamma)^{23}\text{Na}$  ( $E = 439 \text{ keV}$ ) in a Ge(Li) detector and calibrating with a sample of known composition it is possible to detect less than 50 ppm sodium in short macrobeam irradiations (5). For the microbeam irradiations the total accumulated proton charge is 50 to 100 times less than for macrobeam analysis and consequently the estimated detection limits will be approximately 300 to 500 ppm. Since this technique is not sensitive to attenuation it is quite promising for use in combination with the PMP in the analysis of skin. Further experiments in this field will be carried out in the near future.

For magnesium, the second light element of relevance for skin characterization, our present microbeam detection limit in thin samples is around 800 ppm. This value may be improved by using a detector with a thinner window inside the chamber (or a windowless detector).

#### PIXE versus EIXE

EIXE and PIXE are closely related techniques. However there are important differences between the two methods.

Although the EMP can be focused down to a diameter well below  $0.1 \mu\text{m}$  the effectively excited volume has a considerably larger extension due to the pear-shaped form of the excitation volume.



However, in thin sections localization of the electron probe within a subcellular compartment such as a mitochondrion is feasible and makes it possible to detect gradients of elements within the cell envelope. Whereas the EMP has the advantage of a precise localization even within subcellular compartments the PMP can only be focused down to a width of a few micrometres. In some microprobes (22,26) it is possible to work with a resolution of 1 - 2  $\mu\text{m}$ , but with a further limited beam current. For samples not extremely thin, such dimensions are rather competitive to the electron probes since for protons no significant degradation of resolution occurs due to scattering within the sample.

The sensitivity of the EMP system is such that trace elements cannot be determined without certain preparatory measures such as micro-incineration. In applications to biological material the method has an uncertainty in the determinations at a 10 % level. The minimal amount of an element that can be detected is at optimal conditions of the order of  $10^{-18}$  g, the minimal concentration detectable lies in the range 0.02 - 0.2 % depending on the particular element and the type of specimen under analysis. The proton microprobe, on the other hand, has minimal detectable concentrations of the order of 1 - 100 ppm. The quantification technique is absolute and no standards are required, since the number of protons hitting the sample can usually be measured. The uncertainties are normally below the 10 % level. With high beam densities and very small beams (1  $\mu\text{m}$ ) the minimum detectable absolute amount is of the order of  $10^{-16}$  g. When selecting the proper technique great care has to be taken when considering whether very high spatial resolution is required or if a less resolved beam but more sensitive analysis is suitable. Comparative analysis on the same targets from skin and its appendages with both techniques are under way at our laboratories.

### Conclusions

Using a proton beam of millimetre dimensions the longitudinal distributions of many elements in human hair can be determined non-destructively. Thus measures of exposures and deficiencies with a time resolution of less than one week are offered. Such information may also be of interest for forensic investigations and in environmental and occupational health research. In contrast to the electron microprobe which can be used well below 0.1  $\mu\text{m}$  resolution it is with the proton microprobe only possible to attain a spatial resolution of about a few micrometres. This implies a restriction when using a PMP in sub-cellular compartments. However, due to its significantly higher relative sensitivity the PMP may be used instead of or complementary to the EMP in many applications in dermatology.

In this work a radial scan of a thin hair section from a subject with high iron concentration in the domestic water supply has been performed using a proton microprobe. Several interesting results were found regarding the distribution of elements such as calcium, iron and

zinc. A comparison with some other investigations made in thick hair sections shows similar distributions except for the very sharp peak concentrations found in this study.

In a scan of a thin section of normal human skin the elemental depth distributions were in good agreement with those from EMP studies on guinea pig skin. With the PMP method elements like calcium, iron and zinc were detected. With minor improvements of the analytical procedure we estimate that there is a substantial probability of detecting other trace metals at the 5 to 10 ppm level.

Although the material reported in this work is rather limited, it is together with results from other studies discussed possible to establish that the high sensitivity of PIXE analysis makes it a valuable tool in quantitative analysis of dermatological materials.

### Acknowledgements

For excellent preparative work we thank Mrs Eva Björkner. We also thank Dr Magnus Lindberg for help with the skin biopsy.

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#### Discussion with reviewers

V. Valcovic: In all interdisciplinary work there is the problem of correlating the possibilities of the method and the way of thinking of one discipline with the philosophy of research in the other discipline. Could the authors comment on research lines they plan to follow in dermatology beyond data collecting and comparison with the body of knowledge in this field? Using this as a starting point is quite alright.

Authors: The physiological regulation of cell metabolism in single strata of the stratified epidermis is of importance for understanding the reactions leading to skin changes in contact skin reactions. No physiological probe allows detection of changes in, e.g. calcium levels which means that elemental analysis in quench-frozen sections is at present the only acceptable means of assessing elemental redistribution in a single cellular stratum. The electron microprobe is not sensitive enough to allow assessment of changes in calcium and magnesium levels at statistically significant levels.

Consequently, PIXE has been the method of choice in the search for methods allowing such determinations in normal and diseased skin. We expect to be more successful in this respect in the future when we have accumulated more experience in the analysis of skin.

Concerning trace element analysis studies on the distribution of elements in normal hair and in hair from patients with systemic (as well as systemic genetic) disease(s) we are investigating the possibilities of elemental analysis as a resource for precise clinical diagnosis. Studies of hair cross sections will lead to an understanding of the effects of external contamination as contrasted to endogenous up-take.

V. Valcovic: What are the detection limits obtained by the authors, (a) with normal beam, (b) with beam in helium, (c) with microbeam?

Authors: For the single hair strand irradiated in helium gas (b) the detection limit (3 S.D.) for mercury was 8  $\mu$ g/g (peak area: 5 pulses; proton current: 4 nA/mm<sup>2</sup>; hair section length: 2 mm; analysis time: 100 seconds). By scanning the hair back and forth with a speed of 1 mm/second the time can be reduced by a factor of 2 - 3. With vacuum analysis (a) approximately 4 times longer



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time is required to obtain the same spectrum (the helium gas has negligible influence on the spectrum shape).

The detection limits for one of the points in the microbeam hair scan (c) are given in figure 6.

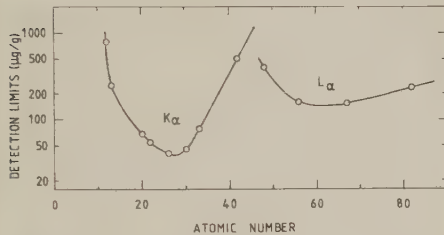


Fig.6. The detection limits for our present microprobe system. The analysis parameters were: time 250 s; current 0.2 nA; count rate 400 pulses/s; detector solid angle 0.0179 sr; sample thickness 0.5 mg/cm<sup>2</sup>. The detection limits are defined as three times the uncertainty of the background in a 2 FWHM region under the peak of interest. The gaussian components of the characteristic peaks are not included in the background.

For a thick sample and equivalent conditions the detection limit for heavier elements ( $Z > 22$ ) would be 10 times lower, however, the energy deposition in the sample would increase by a factor of about 50.

J.A. Swift: It seems remarkable for so many elements in PIXE analysis of hair cross sections (both your work and that mentioned under reference 6) that there is apparently an annulus about 10 µm below the hair surface containing the element at much higher concentration than in the rest of the section. In your own work for example the zinc concentration is nearly one order of magnitude greater in this annulus than at the centre of the hair section. Can this be explained in biosynthetic or structural terms or is it possible that this annulus is the artifact caused by an "edge" effect between the hair surface and the supporting medium?

Authors: This is an interesting question which obviously deserves attention. Several explanations for this non-uniform distribution may be offered. The cortex cells of the outer annular zone are derived from matrix cells low down on the papilla of the root whereas those of the centre of the cortex derive from areas close to or at the tip of the dermal papilla. The different areas of the matrix cells on the papilla may, due to differences in diffusion path distances from the capillary bed of the papilla, have different chances of elemental uptake.

One of the obvious reasons for an uneven distribution may be related to an uneven distribution of the mass of the keratin matrix

which is probably the supporting structure for the incorporated trace elements (metals). As yet we have not correlated the assessed amount of trace elements to the corresponding mass distribution as measured by stereological methods. In our experience we have found that in general the centre of a hair fibre has a much more loose arrangement of the cortex cells than is seen at the fibre periphery. Open intercellular spaces may thus be observed in the centre of the hair fibre whereas the cells of the periphery are closely adjoined leaving no open intercellular voids.

The external contamination is an interesting problem. To the best of our knowledge, so far no systematic study of the barrier offered by the cuticular cells to metal ions has been performed. Such a study also involves the problem of possible cuticular damage due to weathering which may drastically change the barrier capacity of the cuticle. The cuticular cells are definitely permeable to amino-acids such as cystine as previously shown (cf Forslind B. (1971). Electron microscopic and autoradiographic study of s-35-L-cystine incorporation in mouse hair follicles. *Acta Dermatovener* 51, 9-15 and Forslind B, Lindström B, and Swanbeck G. (1971). Microradiographic and autoradiographic studies of keratin formation in human hair. *Acta Dermatovener* 51, 81-88). We do not know if the cuticular cells offer any permeability resistance to metal ions.

The uneven distribution is not an "edge" effect since the supporting medium contained no recorded traces of the elements determined within the hair cross sections. It would, however, be possible to get unreasonably high values of concentrations exactly at the surface of the hair since a high concentration of an element situated at the surface of the hair cannot be normalised to the matrix in a meaningful way.

J.A. Swift: I am concerned about the the very considerable dwell times of the proton beam on the hair during studies of linear distributions of elements (200-500 seconds). Horowitz et al (reference 11) used a method of rapidly scanning the hair about the beam to do this job and to dissipate beam power over a wider area without compromising either spatial or elemental resolution. Have you examined your hairs in the SEM to see whether there is any damage inflicted by the proton beam?

Authors: (see also answer to question 2 by G.J.F. Legge and answer to question 2 by V. Valcovic). When PIXE analysis is applied to single hair strands as described in the text the problem of beam damage is highly significant. Precautions are taken in using helium gas, an excellent cooling medium, in the irradiation chamber during bombardment. We have also used a SEM to examine hair strands irradiated under different conditions. As seen from fig 7 we observe phenomena like "swelling" or "bursting" of the irradiated part when too high current densities are used. It is likely that some elemental losses occur at excessive current densities particularly in vacuum.

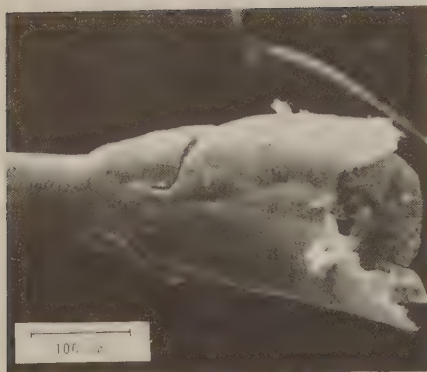


Fig.7. SEM picture of a hair bombarded with 2.5 MeV protons in vacuum; intensity: 2-3 nA/mm<sup>2</sup>. The hair was not broken until it was mounted for the SEM.

For acceptable current densities no changes on the hair was seen in the SEM. The method cited in reference 11 has later been introduced into our irradiation facility and the estimated increase of the maximum current density is about a factor of 3. (cf Li HK, Malmqvist KG, Carlsson L-E and Akseilsson KR. (1983). A PIXE system for routine longitudinal scanning of single hair strands. In: Proceedings of Third Int Conf on Particle Induced X-ray Emission and its Anal Applic, will be published as a volume of Nucl. Instr. and Meth. in Phys. Res. in 1984.)

J.A. Swift: Some of the references you quote (particularly numbers 19,20 and 21) are not generally available outside Sweden. Since the information in them is the key to your work and of general interest can you give a brief synopsis of them? This particularly concerns the special algorithm for determining hair diameter from the particle energy spectra and the principles for calculating surface-to-bulk ratios of elements.

Authors: The references quoted will be submitted shortly (in 1983) for publication in Nucl. Instr. and Meth. in Phys. Res.. In the present work these references are mainly relevant for the minor part concerning longitudinal scans of single hair strands.

In reference 19 it is shown, theoretically as well as experimentally, that the intensity of protons back-scattered from a thin surface layer of the hair, which corresponds to the number of counts between two chosen channels in the observed proton back-scattering spectrum, is linearly proportional to the hair diameter.

In reference 20 the significance of the geometrical hair diameter for calculating correction factors for proton slowing down and X-ray attenuation is shown. In reference 21 a semi-

quantitative method for determining surface-to-bulk ratios of elements is discussed. By analysing with 2 or more different proton energies and comparing the yields of characteristic X-rays from an element at the different irradiation energies it is possible to reveal whether an element is homogeneously distributed in the hair matrix (bulk) or mainly situated at the surface.

J.A. Swift: What is the mean free path of 2.5 MeV protons into hair and skin?

Authors: The total range of 2.5 MeV protons into hair is about 10.8 mg/cm<sup>2</sup> and in skin about 10.2 mg/cm<sup>2</sup>. The difference is mostly due to the different hydrogen contents. In the 1 mg/cm<sup>2</sup> samples in microbeam applications the protons lose approximately 5.5 % of their initial energy and this causes the X-ray production to decrease by 2 to 4 % from the front to the back surface.

G.J.F. Legge: In elemental microanalysis of biological tissue, extreme precautions must usually be taken to preserve not only the morphology but more importantly the elemental integrity of the sample. Although hair was quenched and cryosectioned, it was first swelled in distilled water and later thawed and air dried - all processes of some risk with most biological tissues. What were the justifications for these treatments of this tissue?

Authors: The cross sections of the hair studied were put dry into the embedding medium immediately before the freeze-quenching and subsequently freeze-sectioned (We have unfortunately expressed ourselves vaguely - it is the carboxy methyl cellulose which is swelled in distilled water). Effectively the sections were freeze-dried in the cryo-chamber for approximately half an hour before they were fixed to the Kimfol foil at ambient temperature. Seen in the preparative microscope the thawing involved no detectable melting of ice. We are therefore confident that thawing has little effect on the elemental distribution in hair cross sections. However, it must not be inferred that this particular preparation method can be applied to other tissues which are not dehydrated in the natural state as are hair fibres.

G.J.F. Legge: Mention was made of reducing detection limits partly by increasing current density. The problem of beam heating was also mentioned. What evidence is there concerning the ability of hair, in vacuum or in helium atmosphere, to withstand bombardment by high density proton beam, particularly in spot (unscanned) mode? Is there any evidence on possible losses of elements or mass from the samples during bombardment? How repeatable were the small variations in sulphur distribution mentioned for the cross sectional hair scan?

Authors: (See also answer to question 2, J.A. Swift) In order to increase current density in longitudinal hair scans it is necessary to perform analysis in rapid scanning mode. Such a system is at present being installed in our irradiation facility. According to preliminary tests the current density can be raised by a factor of 3 in scanning mode relative to the stationary mode (Li

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HK, Malmqvist KG, Carlsson L-E and Akseilsson KR, (1983). A PIXE system for routine longitudinal scanning of single hair strands. In: Proceedings of Third Int Conf on Particle Induced X-ray Emission and its Anal Applic, to be published as a volume of Nucl. Instr. and Meth. in Phys. Res. in 1984).

Since our microbeam facility is not equipped with a beam scanning system we have deliberately been using moderate current densities (normally 0.15 nA on a  $4 \times 15 \mu\text{m}^2$  area). We have not observed any signs of long-term losses of mass or elements. Systematic tests of possible elemental losses have been carried out by Legge (Legge GJF, In: The Scanning Proton Microprobe. Microbeam Analysis - 1980, San Fransisco Press Inc 1980). He used an extreme current density (10 nA on a circular beam area with a  $12 \mu\text{m}$  diameter) on a  $5 \mu\text{m}$  thick section of biological matrix in an unscanned mode. The results show no significant losses of mass but very rapid (within a few seconds) losses of some elements, e.g. chlorine. Another systematic long-term test of beam effects on hair sections was carried out by Bos et al (Bos AJJ, van der Stap CCAH, Valcovic V, Vis RD and Verheul H. On the incorporation of trace elements into human hair measured with micro-PIXE. In: Proceedings of Third Int. Conf. on Particle Induced X-ray Emission and its Anal. Applic., to be published as a volume of Nucl. Instr. and Meth. in Phys. Res. in 1984). With a one-hour irradiation in unscanned mode at  $0.5 \text{ pA}/\mu\text{m}^2$  (beam area  $5 \times 12 \mu\text{m}^2$ ) on a  $30 \mu\text{m}$  thick section of hair no significant losses of the elements S, Cl, Cu and Zn were detected.



## PROTON AND ELECTRON MICROPROBE ANALYSIS OF HUMAN SKIN

K.G. Malmqvist<sup>1</sup>, L.-E. Carlsson<sup>1</sup>, B. Forslind<sup>2</sup>, G.M. Romans<sup>3</sup>  
and K.R. Akselsson<sup>4</sup>

1. Department of Nuclear Physics, Lund Institute of Technology,  
Sölvegatan 14, S-223 62 LUND, Sweden
2. Department of Medical Biophysics, Karolinska Institute,  
S-104 01 STOCKHOLM 60, Sweden
3. Wenner-Gren Institute, University of Stockholm, Norrtullsgatan 16,  
S-113 45 STOCKHOLM, Sweden
4. Department of Work Environment Technology, Lund Institute of  
Technology, Box 725, S-220 07 LUND, Sweden

In order to demonstrate the feasibility of the proton microprobe in the analysis of dermatological material when a spatial resolution of a few micrometres is sufficient and to compare it with the electron microprobe technique, duplicate sections of human skin have been analysed with both methods.

A skin sample was obtained from each of three healthy volunteers. After cryosectioning (12  $\mu\text{m}$ ) and freeze-drying adjacent sections of each sample were scanned by the electron microprobe and the proton microprobe respectively.

### 1. INTRODUCTION

Biochemical and biophysical methods of determining the composition of skin and its appendages have existed for many years but only recently, through the introduction of the electron microprobe (EMP) into dermatological research (1,2), has information on elemental concentrations in situ at the cellular or subcellular level been available. The high spatial resolution of the EMP has made it a well established and versatile instrument for studies of elemental composition of biological structures (3). However, due to the intense background in the X-ray spectrum from electron bremsstrahlung EMP analysis is limited to elemental concentrations above about 0.1 per cent.

To extend the number of elements that can be analysed in situ in dermatological samples the proton microprobe (PMP) may be used as a complementary instrument when a spatial resolution of the order of a few micrometres is sufficient. The higher relative sensitivity of PIXE as compared with EIXE (Electron Induced X-ray Emission) in combination with high precision and accuracy has lead to a rapid development of PMP-facilities. The properties and applications of PMP systems are extensively reviewed by, e.g. Cookson (4) and Martin (5).

Several research groups have presented results from PMP analysis of skin and its appendages. Cookson and Pilling (6) and Bos et al. (7) made cross-sectional scans of thick slices of human hair. Results of great interest in the interpretation of data from whole hair analysis were obtained. Bischof et al. (8) reported depth profiles in human skin showing the presence of a hair root by strong elemental variations. Malmqvist et al. (9) reported some preliminary results from scans of thin sections of quench-frozen, freeze-sectioned and freeze-dried normal human hair and epidermis.

The aim of the present paper is to demonstrate the applicability of the proton microprobe in dermatological research and to compare the analytical characteristics with those of the well established electron microprobe.

## 2. MATERIALS

Skin samples were obtained by punch biopsy from the upper arms of three healthy volunteers. The biopsies were quench-frozen in freon, cooled by liquid nitrogen at approximately  $-190^{\circ}\text{C}$  and then placed in a Minotome cryostat (International Equipment Co) at  $-25^{\circ}\text{C}$  where four sections (approx.  $12\text{ }\mu\text{m}$  thick) were cut perpendicular to the skin surface. The sections were freeze-dried in the cryostat for 22 h. The samples intended for subsequent PMP analysis were mounted between two supporting foils (Kimfol R). For EMP the duplicate samples were mounted on spectrografically pure carbon sheets (1 mm thick). To prevent uptake of moisture prior to analysis samples were stored over Drierite.



### 3. METHODS

#### 3.1 Proton microprobe

For the PMP analysis 2.5 MeV protons from a tandem electrostatic accelerator (Pelletron 3UDH) were used. An adjustable object collimator is situated in the beam line after the analysing magnet at a distance of 4 m from the irradiation chamber. The collimator can be set from 10 to 1500  $\mu\text{m}$  in two perpendicular directions. Four quadrupole magnets in front of the samples can be used to focus the beam to dimensions of a few micrometres. In this experiment only 2 magnets were used in a doublet configuration with an object aperture of  $100 \times 300 \mu\text{m}^2$  and the 90 % size of beam spot obtained on the sample was approximately  $5 \times 100 \mu\text{m}^2$  (fig 1) with proton current densities of 1 to 2  $\text{pA}/\mu\text{m}^2$ .

The skin samples were mounted on a holder capable of taking eight samples and movable by remote control in increments of 1  $\mu\text{m}$  or more in horizontal and vertical directions. The rectangular beam spot was oriented with the long side parallel to the skin surface, starting in the dermis and then the sample was moved in 4  $\mu\text{m}$  increments to analyse the various epidermal strata (fig 1).

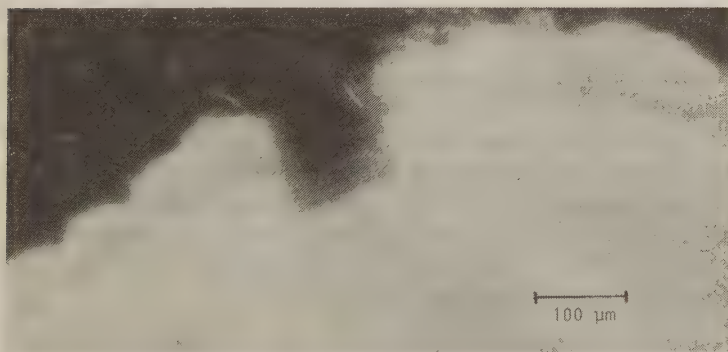


Figure 1: Micrograph of specimen (sample 3, section 1) after proton bombardment.

The total accumulated charge in each analysis was 0.1  $\mu\text{C}$ . In fig.1 a micrograph from one of the irradiated samples is shown. The characteristic X-rays were detected in a Si(Li) detector (Kevex, 80 mm<sup>2</sup> internally collimated to 60 mm<sup>2</sup>, FWHM 160 eV at 5.9 keV) positioned at an angle of 135 degrees relative to the beam. For the light elements (Z<20) corrections were made for the absorption of X-rays within the sample.

### 3.2 Electron microprobe

An electron microscope (JEOL 100C) provided with a JEOL ASID-4B scanning attachment and a Kevex energy dispersive spectrometer (10 mm<sup>2</sup> Si(Li), FWHM 146 eV at 5.9 keV) was used for the EMP analysis in the SEM mode with a stationary spot and a narrow beam (< 100 nm). The accelerating voltage was 20 kV, the specimens were tilted 35 degrees and the counting times were 80 s in each spot. The effective spatial resolution is restricted by the lateral scattering of electrons within the sample and is about 10  $\mu\text{m}$  in the present case. The effective depth of X-ray production is about 12  $\mu\text{m}$  (10).

Analyses of three spots separated by a distance of approximately 50  $\mu\text{m}$  were made at each level parallel to the skin surface. The elemental concentrations calculated as the average of these three determinations were used for comparison with the elemental concentrations obtained from the PMP.

For quantification standard samples made of salts ( $\text{NaCl}$ ,  $\text{KH}_2\text{PO}_4$ ) dissolved in a matrix consisting of a mixture of 5 % glycerol and 20 % gelatin in water were used. The standard gel was frozen, sectioned and dried in exactly the same manner as the actual skin specimens and mounted on the same type of carbon support. Peak-to-background ratios for the elements detected were used for quantitative analysis (11).

## 4. RESULTS AND DISCUSSION

From each of the three skin biopsies (1 to 3) four adjacent sections (a to d) were cut of which a and c were taken for PMP analysis and b and d for EMP analysis. During bombardment corresponding parts of the sections were analysed with both methods.

#### 4.1 Proton microprobe analysis

When calculating the elemental concentrations from the PMP X-ray spectra the sample mass thickness has to be known. In the present work the known thickness of the supporting foil ( $180 \mu\text{g}/\text{cm}^2$  as obtained by weighing) and the linear relation between sample thickness and secondary electron bremsstrahlung (SEB) intensity (12) for the thickness interval of interest in this work ( $<2 \text{ mg}/\text{cm}^2$ ) has been utilized. In the spectra from the skin samples in question the region 4 to 6 keV is almost completely free of characteristic peaks. The sample thicknesses of sections a and c of sample 1 are plotted in fig.2 versus position on the skin section as determined by integrating the SEB in this energy region and normalizing to the SEB from the supporting foils assuming similar matrix composition. The nominal setting of the cryotome (i.e.  $12 \mu\text{m}$ ) is not expected to correspond exactly to a constant real thickness of the sections produced. Although the two sections in fig.2 are separated by a distance corresponding to the thickness of one section as described above and the absolute thicknesses seem to differ somewhat, the agreement between the shapes of the mass distributions of the two sections is very good.

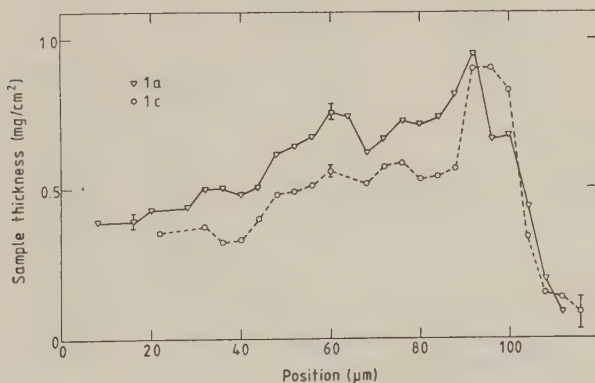


Figure 2: Sample mass thickness plotted versus position on the skin section for the two duplicate sections from sample 1. The distinct decrease in the thickness at the far left corresponds to the skin surface. The lines are drawn to guide the eye and the error bars show the statistical uncertainties ( $\pm$ one S.D.).

Several light elements of relevance to dermatology are present in rather high concentrations. In fig.3 are plotted the concentration distributions of phosphorus, sulphur, chlorine and potassium for both sections of sample 1. The distributions in the two duplicate sections show very good agreement considering the normal biological variability. This demonstrates the good reproducibility of the PMP method.

As expected phosphorus increases drastically in the lower part of the epidermis, stratum germinativum, where the cells are formed. The concentration then slowly decreases in the suprabasal levels, where phosphorylated precursors to keratins are dephosphorylated (13), to fall off sharply when entering the stratum corneum where the phospholipids are replaced by ceramides to form the barrier of the horny layer (14). Also the elements chlorine and potassium vary significantly in the various epidermal strata while the sulphur concentration is rather constant in relation to the total mass except for a minor increase in the stratum corneum.

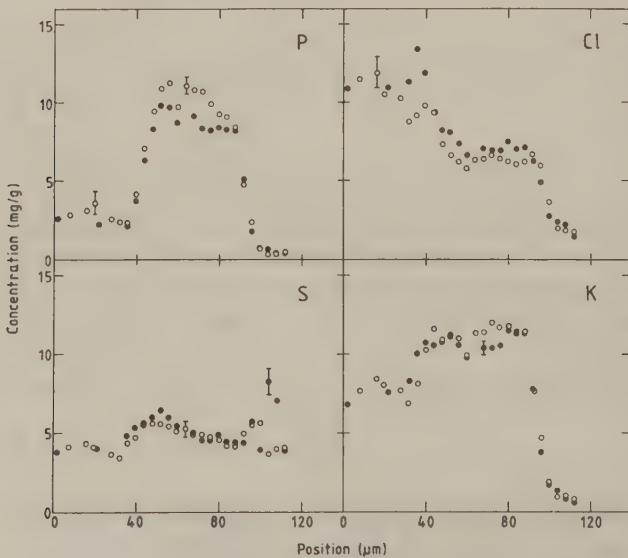


Figure 3: Elemental distributions in sample 1 section a (o) and c (●). The error bars show examples of the estimated uncertainties.

The inter-subject variations for phosphorus and chlorine levels are shown in fig.4 where the elemental concentrations are plotted versus the position on the sections. To facilitate this comparison the distributions have been plotted by matching the position of the "leading edge" of the phosphorus distribution in one section to the others. The plots demonstrate the variation in thickness of epidermis but on the whole elemental distributions as well as the absolute concentrations are very similar in all three samples.

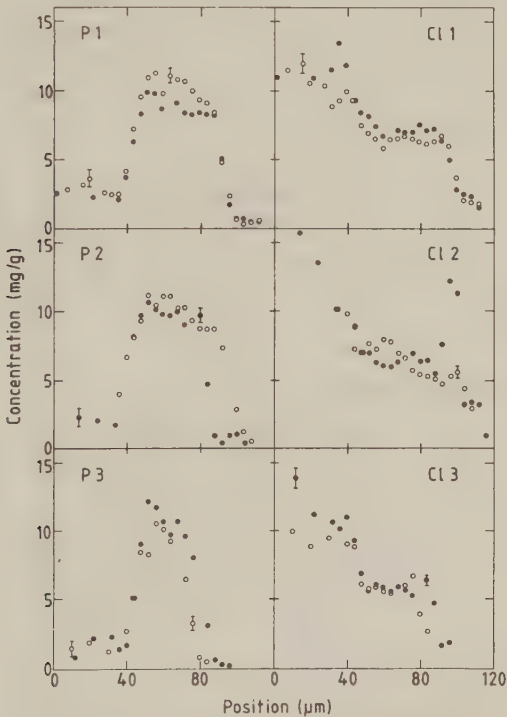


Figure 4: Phosphorus and chlorine distributions in all three skin samples (1 to 3) for sections a (o) and c (●). The error bars show examples of the estimated uncertainties.

Some heavier elements occurring in concentrations below 500 ppm are important to the study of the physiological processes in normal and diseased human epidermis. Furthermore the transport of potentially toxic elements, e.g. chromium and nickel through the protective barrier into the epidermis is a very interesting field in, e.g. occupational dermatology. Thus it is important to find ways of detecting these elements and the high relative sensitivity of the PMP will then be very useful. In figs.5 a to c plots are made of the calcium, iron and zinc distributions in all sections analysed by the PMP. The plots have been matched to the steep increase of phosphorus concentration (broken line) in each section in a similar way to those in fig.4. It is clear for all sections that iron and zinc peak in the stratum germinativum and decrease towards the skin surface. The calcium distributions may reflect an exogenic contribution from domestic water through the horny layer.

In figs.5 b and c data points corresponding to sections a and c of sample 1 are connected to show the variation between duplicate sections from the same skin sample. Despite poor pulse statistics, it is evident that quite reproducible distributions are obtained for both elements.

#### 4.2. Electron microprobe analysis

For three skin sections comparative analyses were performed using the EMP. The special advantage of high spatial resolution obtainable in very thin sections was not utilized in this experiment. A resolution of a few micrometres is sufficient to study intracellular elemental variations and thus thicker samples giving increased sensitivity were used. The standards used for quantification were analysed under the same conditions as the skin samples.

#### 4.3. Comparison between the proton and electron microprobe

##### 4.3.1. Quantification procedure

In fig.6 is shown a direct comparison between the elemental distributions obtained in EMP and PMP analysis respectively. In the EMP analyses the positioning of the beam is made by identifying the various epidermal strata in the SEM display. This introduces a small



uncertainty when referring to the scale of the abscissa in fig.6. The estimated uncertainty is indicated by the horizontal error bars.

As seen from the diagrams the general shapes of the elemental distributions are rather similar for both modes of analysis.

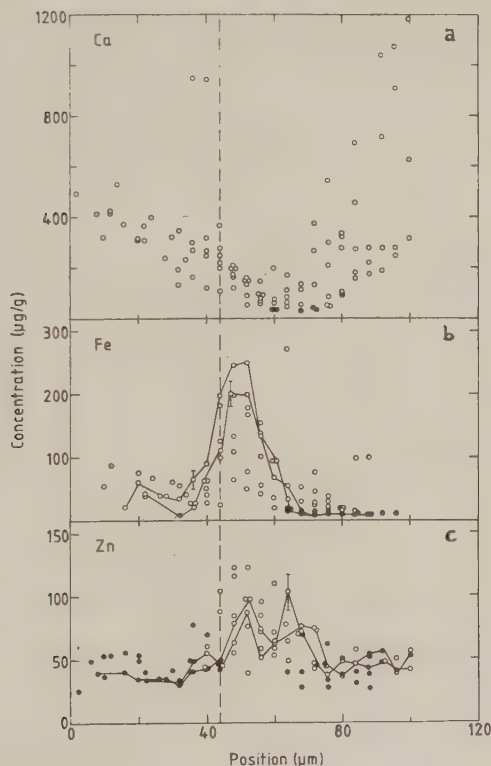


Figure 5: Trace elemental distributions plotted for a) calcium, b) iron and c) zinc, versus position for all sections with position related by matching the position of the steep phosphorus increase in each section to the position in one section (indicated by broken line). Values below the detection limits are represented by filled circles at the detection limits. The solid lines in b) and c) connect corresponding values from section a and c of sample 1. The error bars show the statistical uncertainties ( $\pm$ one S.D.).

However, some systematic differences exist in the quantitative results of the two methods yielding concentrations up to 30 per cent higher in the EMP case. Irreproducible mass losses under electron beam irradiation may cause errors in quantification. Part of the difference may also be explained by biological variability and difficulties in positioning the beam spots completely equivalently in both techniques. The relatively smaller part of the sections analysed in EMP and irreproducible mass losses may explain the greater variation between duplicate samples in EMP as compared with PMP analysis.

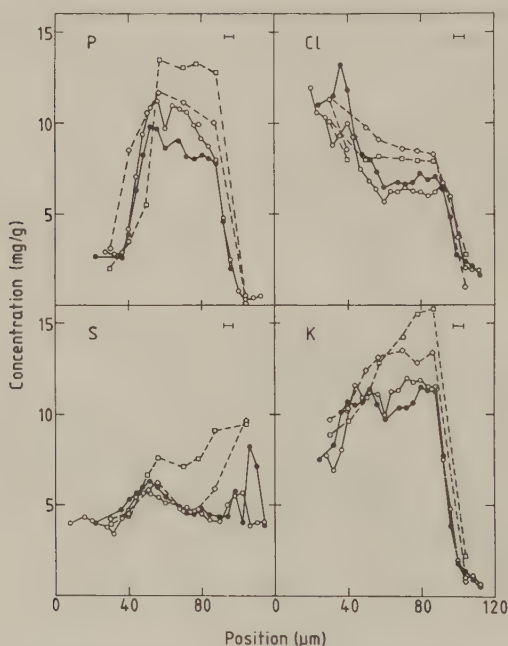


Figure 6: Comparison of the elemental distributions determined using the PMP (solid lines) and the EMP (broken lines). The lines are drawn to guide the eye and error bars indicate the uncertainty in position of the EMP in relation to the scale used for the PMP determination.

This peak-to-background approach in EMP quantification will take into account varying density and composition of the matrix as well as the influence from surface roughness. In the PMP analysis inhomogeneities of the supporting foils would directly affect the elemental levels. Such errors can be estimated to be less than 10 per cent. The statistical uncertainty in the thickness determination is rather high and also with varying matrix composition there is an uncertainty due to variation in SEB intensity. An improved thickness determination can be performed by measuring the energy distribution of protons back-scattered from the sample (15). Also in the case of PIXE it is possible to use the peak-to-background approach and normalization to standards for quantification. This would be particularly beneficial in analysis of the very light elements in reducing the effect from surface roughness on concentration calculations.

#### 4.3.2. Elemental range

The possibility of determining the lightest elements, i.e. sodium and magnesium, is in all X-ray methods restricted by the attenuation of X-rays between specimen and detector. In the special experimental arrangements used in this work the EMP has the highest transmission thus extending analysis down to sodium. Quantification at the low-energy end of the spectrum is, however, difficult in the case of non-flat specimens. Using thinner windows in PIXE analysis it is also possible to detect elements with  $Z < 15$ . The use of a lower proton energy will improve the detection limits for the lighter elements.

For elements present close to trace levels (10 to 500 ppm) the EMP is of limited value with detection limits in the range of 0.2 to 2 mg/g depending on the particular element and type of specimen under analysis. However, from the results in the present work it is concluded that the PMP can produce unique information on such elements in situ on cellular or subcellular level in human skin.

#### 4.3.3. Spatial resolution

As earlier discussed the highest attainable spatial resolution of the EMP requires extremely thin specimens, i.e. 100 nm. In the present case of semi-thick specimens the spatial resolution is difficult to define unequivocally since it depends on the volume of effective X-ray

production and hence on the elements analysed. In the biological matrix it is of the same order as the depth of penetration of the electrons (10-12  $\mu\text{m}$ ). Due to sample thickness, the spatial resolution of the PMP is in the present case equivalent or even somewhat higher than that of the EMP. However, a high spatial resolution, e.g. in order to analyse small subcellular structures, is attainable in the EMP method by reducing the section thickness. In the case of the PMP the resolution is limited by the quality of the beam optics used and is with present technology always larger than about 1  $\mu\text{m}$ .

#### 4.3.4. Beam effects

As has been discussed by Cookson (16) the energy deposition per characteristic X-ray is under normal conditions higher in the PMP than in the EMP analysis. However, the beam intensities used for EMP are often very high (see section 4.3.1.) which may be a problem in some applications to biological material. To reduce target deterioration in PMP analysis with higher current densities scanning of the proton beam may be used. Heck and Rokita (15) demonstrate this when analysing thin sections of stomach mucosa using very high beam current densities (approx. 90  $\text{pA}/\mu\text{m}^2$ ), still keeping target deterioration at a reasonable level.

In applications of the proton microprobes to dermatological samples of approximately 1  $\text{mg}/\text{cm}^2$  thickness, current densities below 2  $\text{pA}/\text{mm}^2$  seem to give only slight sample deterioration in 10-minute bombardments. This is further supported since no signs of melting are observed in the supporting plastic foils. With the rectangular beam spot oriented parallel to the epidermal layers a total proton current of 200 pA resulted in a count rate of 600-800 pulses per second. The basal cells in the skin sections are of the same size as the sample thickness and organized in straight rows parallel to the skin surface. The long narrow beam spot facilitates the simultaneous analysis of the same part of several equivalent cells thus producing data relevant for the dermatologist.

If the beam used for this experiment were to be scanned fast and perpendicular to the surface, a beam current of around 5 nA could be used while maintaining low sample heating. In combination with a suitable hole-absorber the detection limits for heavier elements would

then drastically improve.

## 5. CONCLUSIONS

The proton microprobe has been shown to produce reproducible results equivalent to those of the electron microprobe when applied to human epidermis. The possibility of analysing elements present at levels of 10 to 500 ppm makes the proton microprobe a very powerful tool in exploring new fields in dermatology.

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